



LAB ONE: SPIKES FOR ALL!

A BEGINNER'S GUIDE TO THE SPIKERBOX

OVERVIEW

In this lab you will:

1. learn how to prepare a cockroach leg for electrophysiological readings;
2. observe and record action potentials generated in a cockroach leg using a SpikerBox and a computer; and
3. investigate how different types of stimulations affect the generation of action potentials in a cockroach leg preparation.

OBJECTIVES

Before doing this lab you should understand:

- the purpose and shape of an action potential
- the basics of electrophysiology
- how measuring action potentials from either inside or outside of a cell affects the data collected
- the basic anatomy of the cockroach leg and nervous system

After doing this lab you should be able to:

- distinguish between intra- and extracellular recordings
- perform basic analysis of electrophysiology data
- design basic electrophysiology experiments to study the nervous system of insects
- understand how sensations enter into the nervous system
- explain how an organism can differentiate between multiple types of sensory stimulations
- describe the concept of rate coding

EQUIPMENT

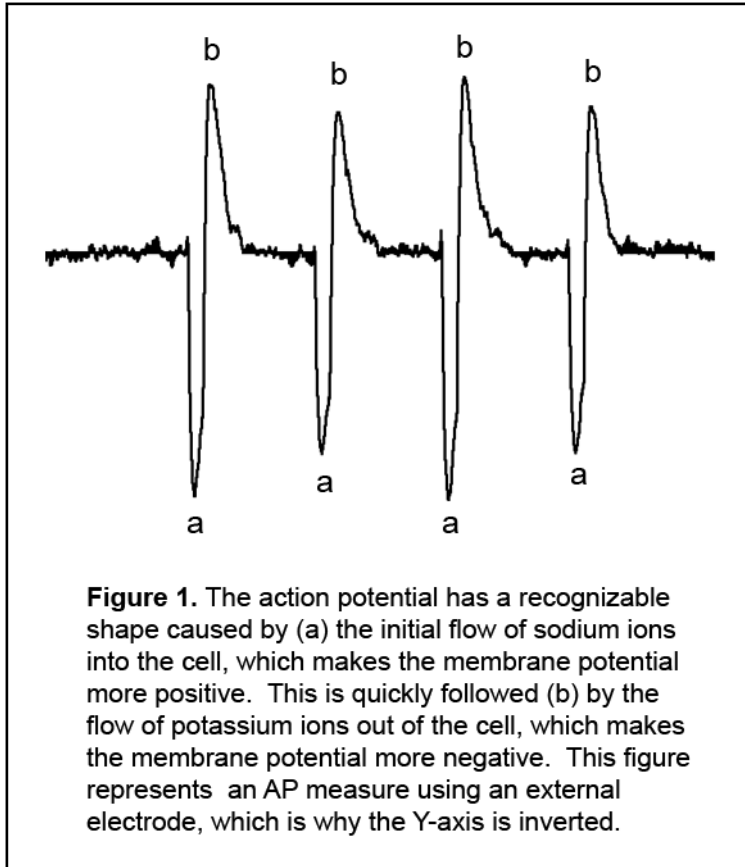
- SpikerBox with audio cable adapter
- cockroach
- ice water or access to a freezer
- dissection scissors
- toothpicks
- drinking straw

INTRODUCTION

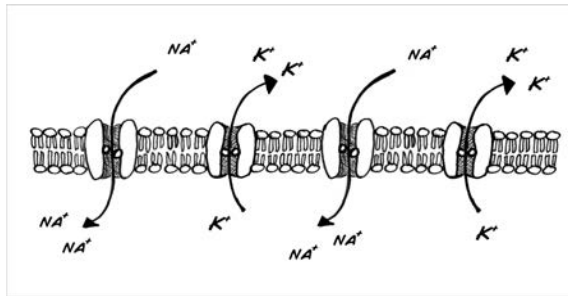
Action Potentials: An action potential (AP) is an electrical event in a small section of cell membrane that acts as fast moving, long-range signal. The AP, once initiated, will spread down the membrane of a cell, thus allowing a cell to communicate with astonishing speed (80 m/s). Neurons can send APs, or spikes, in specific patterns down their axon to convey information at a synapse, the point at which one neuron communicates with another through chemical signaling.

The main feature of an AP is the distinctive shape (Figure 1). This graph shows the charge across a section of membrane over a very short period of time. When a cell is at rest, or not sending an AP, it will be at resting potential. Resting potential is the resulting voltage established by a cell concentrating various ions (Na^+ , K^+ , Cl^-) on different sides of its membrane. This uneven distribution of ions creates a negative charge on the inside of the membrane.

If a neuron is stimulated enough it will generate an AP. During the AP, the membrane has a fast rise and fall in potential caused by the movement of Na and K across the membrane, respectively. The movement of these ions can be measured using electrophysiology.



Synapses: Synapses are the point of communication between two neurons. While an AP is an electrical signal that allows a neuron to communicate along the length of its axon, it is not a signal that can jump from one cell to the next. This requires a neuron to convert the electrical signal to a chemical one in order to successfully communicate. The location of this event is the synapse.



View of Neural Membrane showing flux of ions through channels.

In a synapse, a neuron sending the signal, the presynaptic cell, will stimulate a receiving neuron, the postsynaptic cell, by placing a chemical signal into the synapse. Called a neurotransmitter, this chemical signal will either excite or

inhibit the postsynaptic cell. You may already know several neurotransmitters. Several well-known

ones secreted by the presynaptic cell are glutamate, GABA, dopamine, serotonin, and acetylcholine (ACh). The postsynaptic cell has the specific receptor for the neurotransmitter, which causes changes in electrical excitability once bound.

Electrophysiology: Electrophysiology, the study of the electrical properties of cells, allows researchers to study how neurons communicate with each other and form complex neural networks. In particular, scientists and physicians use electrophysiology to measure the properties of APs in living neurons. Two of the most common approaches used to study APs are to record from either an intracellular or extracellular perspective.

Intracellular recordings allow someone to measure either the voltage (charge) or current (movement of ions) across the membrane of a single cell. This technique requires the insertion of a recording electrode, housed inside a hollow glass pipette, into the membrane of a neuron. A second electrode, the ground electrode, is placed extracellularly. The difference in electrical activity (voltage or current) between the intracellular recording and extracellular ground electrodes reflects the electrical activity of the neuron being studied. For instance, an AP measured using this technique will look like those described in textbooks, with an initial positive depolarization followed by a negative repolarization and overshoot. It is important to remember that intracellular recordings are measuring the change in electrical properties across the cell membrane from the perspective of being inside the cell. This technique can be difficult to perform as the neuron impaled with the glass pipette cannot be moved more than a few microns without damaging or even killing the cell.

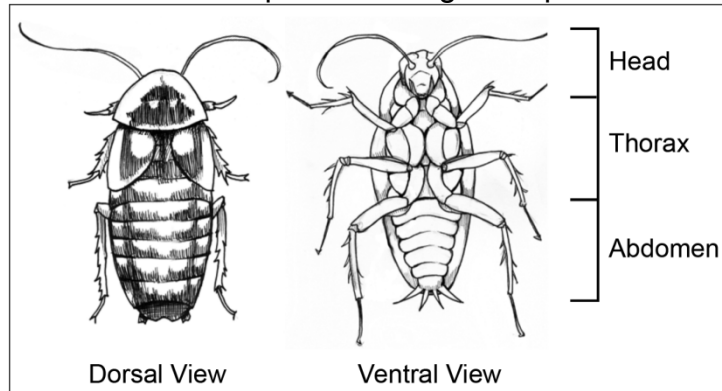
Extracellular recording is a second electrophysiological technique that measures the voltage and current across the membranes of neurons. However, rather than having to insert an electrode into a single cell, extracellular recordings can be made by simply placing a recording electrode adjacent to a cell membrane. For these experiments, measurements of charge and movement of ions across cell membranes will appear to be inverted compared to intracellular recordings. This is because the recording electrode is measuring ions entering and leaving the extracellular space.

Another important way in which data collected from intra- or extracellular recordings differ is the amplitude of APs collected. APs collected from intracellular recordings are consistent in their amplitude during an experiment.

This is because there is little difference in how APs look once they are initiated.

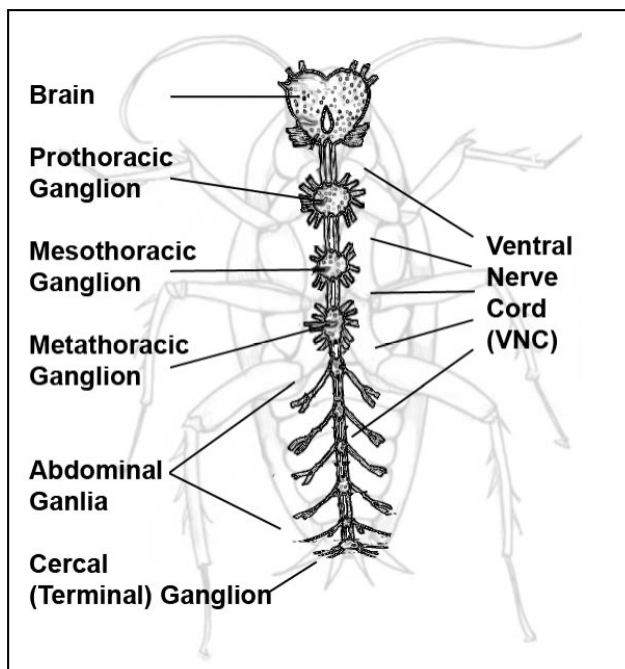
However, the amplitude of APs collected from extracellular electrodes can be of varying sizes for several different reasons.

The first is that extracellular recordings may be measuring electrical activity from multiple cells at one time. If multiple axons near an extracellular electrode are sending APs down their axons, the resulting data will be larger. Additionally, larger axons can also increase the amplitude of a measured APs in an extracellular setup. However, keep in mind that the AP recorded by an extracellular electrode will get smaller the farther away the axon is from the point of measure. The SpikerBox is used to perform extracellular recordings.



Cockroach Anatomy and Senses:

To observe the anatomy of a cockroach, first anesthetize one by placing it in a glass of ice water until it is no longer moving. The anatomy of the cockroach is exceptionally accessible to electrophysiological experimentation for a variety of reasons. First, from the dorsal, or top, view the cockroach has a distinctive prothorax (the section directly behind, and shielding the head) and wings that give the cockroach its distinctive armored look. When flipped on its back, the ventral aspect of the cockroach reveals the basic segmented body sections

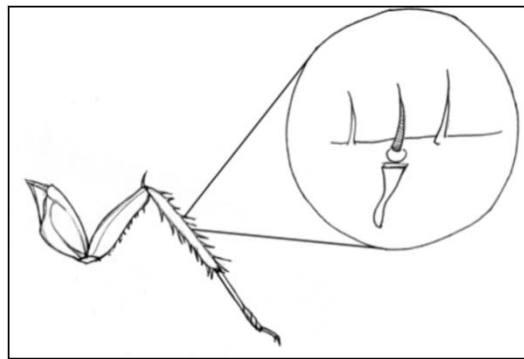


distinctive of insects: the head, thorax, abdomen, and legs. Identify the major delineations between these sections, and observe the segmentation inside these major regions.

For this lab, you will be removing a leg from the cockroach's mesothorax, the last and largest leg closest to the abdomen. The major benefits of this approach are that the leg will grow back, and the cockroach nervous system provides wonderfully large APs that can be observed using the

SpikerBox. Each segment of the cockroach contains a region of the Ventral Nerve Cord (VNC), a collection of neurons that send information to the muscles of the body, while receiving information from the sensory organs of the periphery. This information is relayed to and from the brain using action potentials and synapses. We look to measure these communications entering from the legs of the cockroach.

When observed up close, you can see how the cockroach leg is covered with large spines along the tibia and femur. Each spine has a neuron wrapped around it, which sends APs to the VNC and eventually the brain. The pattern and frequency of APs sent will allow the VNC to distinguish a strong external stimulus from a weak one. Which hair cells are being stimulated will determine where the cockroach perceives the stimulation is located.



SETUP

Computer Setup

Use of a computer for the basic aspects of this experiment is not required, as the SpikerBox is equipped with a small speaker. However, in order to perform some of the analysis described in this manual, a computer will be necessary.

There are several free options for capturing and analyzing data from your SpikerBox.

Backyard Brains iPhone/iPad App: Backyard Brains iPhone/iPad app, which can be downloaded via iTunes. This app can be found at the following link:

[Backyard Brains iPhone/iPad app](#)

To use this app, you will need to purchase or make an audio cable to link the SpikerBox to the iPhone.

Audacity: Audacity is a freeware program designed for editing and recording sounds. While not the original purpose of the software, Audacity is a perfect way

to convert the amplified signal of the SpikerBox into visual and recordable data. Audacity runs on both Windows, Mac OS X, and Linux/Unix. The website for Audacity can be found at the following link:

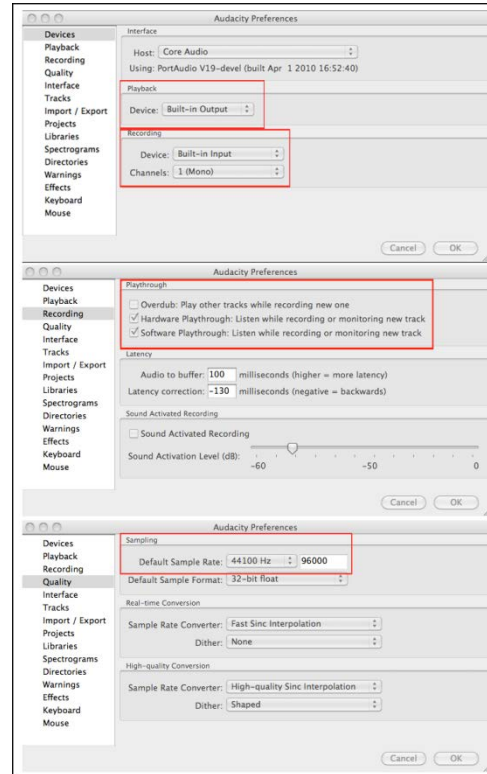
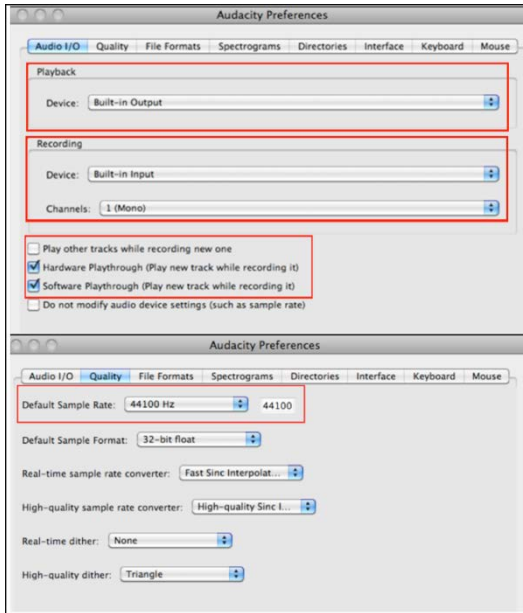
[**Audacity Homepage**](#)

Once Audacity has been installed, refer to the following procedure:

Audacity Setup

1. If using a laptop, fully charge your battery and record from the SpikerBox while unplugged. When plugged in, the SpikerBox will amplify the current running through the electrical system of the building. The result will be an increase in electrical noise recorded by Audacity. Running off of battery power will decrease the amount of electrical noise.
2. If using a laptop, set the brightness of your screen to maximum. Similar to the effects of the surrounding electrical wiring on your recordings, a dimly lit LCD display will create a significant amount of noise that can be amplified by the SpikerBox.
3. Plug your SpikerBox into the Laptop with male-to-male audio cable. This is exactly the same as a headphone cable, except it has “male” connector on each end.
4. Open Audacity, go to **File → Preferences**. Note: Due to differences in Audacity between versions, there may be differences in how the **Preferences** window is organized. The primary settings you need to set up are the same, even if they look a little different on your screen.
5. Select the **Audio I/O** tab in the **Preferences** window. Some versions place this window in the **Devices** tab.
6. Select the **Built-in Input** as the **Recording Device** from the drop down menu.
7. Select **1 (Mono)** from the **Channels** drop down menu.
8. Select **Built-in Output** as the **Playback Device** from the drop down menu.
9. The following steps are found either in the **Audio I/O** or **Recording** tabs in the **Preferences** window. The following checkboxes are listed in order as they appear:
 - **Uncheck** the box: Play other tracks while recording a new one.
 - **Check** the Box: Hardware Playthrough
 - **Check** the box: Software Playthrough
10. Select the tab labeled **Quality**. Set the **Default Sample Rate** to 44100 Hz (or 44,100 times per second).
11. Audacity should now be configured to record from your SpikerBox.

Below are examples of how your **Preferences** window should be configured. Keep in mind that your version of Audacity may look different, but the settings will be the same. Two examples are provided, with the important settings highlighted.

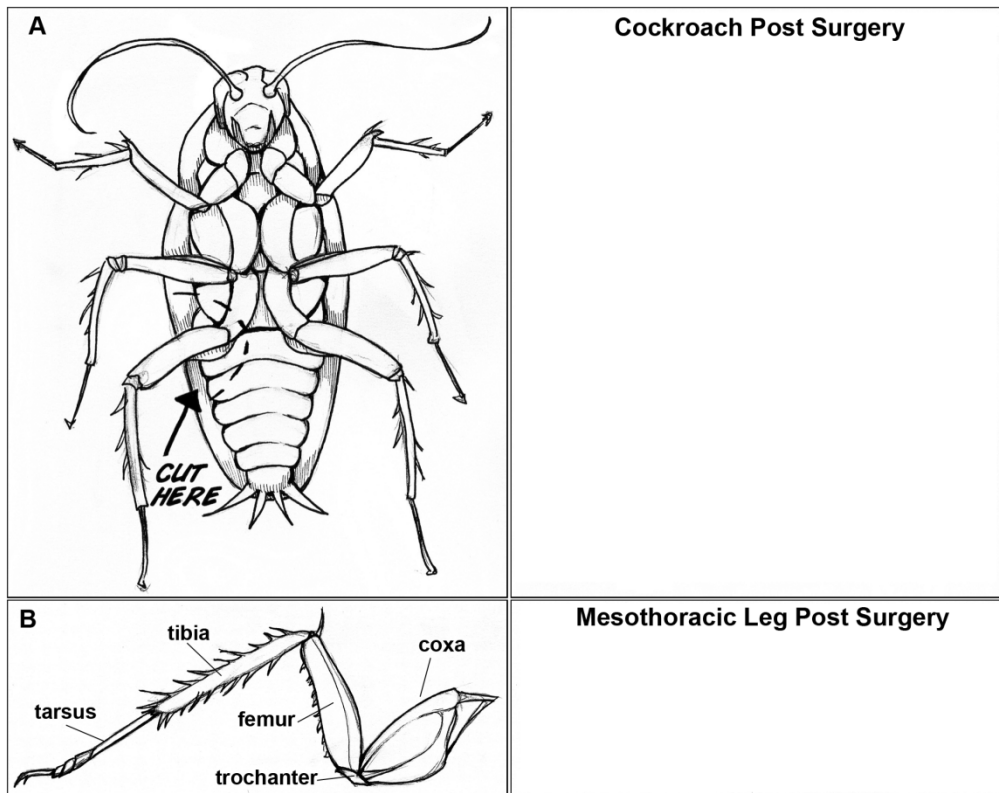


PROCEDURE

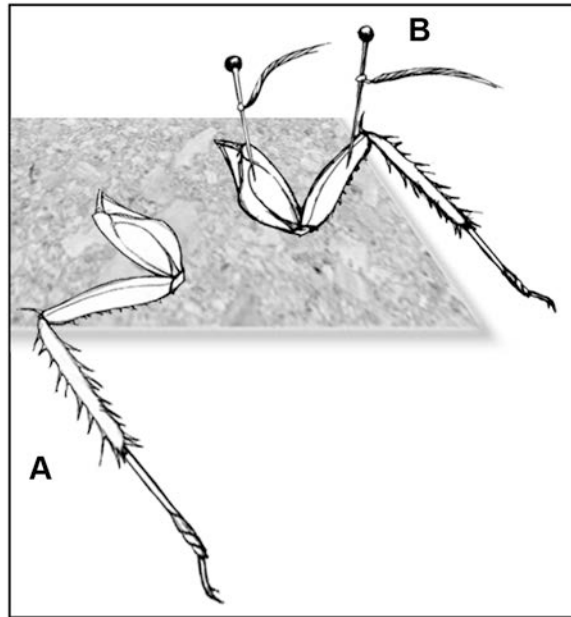
Exercise 1: Cockroach Mesothoracic Leg Preparation

This exercise will teach you how to perform a basic experimental setup used to record spikes in a cockroach preparation.

1. Take a cockroach and either put it in ice water or a standard freezer to anaesthetize it. Wait 5-10 minutes, or until the cockroach stops moving. Take care to monitor how long the cockroach has been placed in the water or freezer, as extended exposure to low temperatures can be fatal.
2. Once your cockroach has been anaesthetized, remove it from the water or freezer and place it on your lab bench. Using dissection scissors cut off one of the mesothoracic legs (see Figure 6A below). Make sure you cut close to the thorax (body) so that the coxa remains attached (Fig. 6B).
3. Put some petroleum jelly (or low temp wax) on the exposed wound of the leg and the accompanying spot on the cockroach body.
4. In the space provided below, sketch your cockroach and the leg you have removed. Note any damaged parts if applicable.



5. Return your cockroach to its housing. The leg will grow back if the cockroach is not a full-grown adult yet. One way to distinguish the age of your cockroach is to note whether the wings are fully formed. Adults have wings while younger nymphs do not.
6. Place the leg on the cork found on top of your SpikerBox. Make sure the coxa and femur of the leg are on the cork, while the tibia and tarsus hang freely (Fig 8A). Insert the electrodes through the leg and into the cork. Insert one electrode into the coxa and the second electrode into the femur (Fig 8B). The electrodes will measure action potentials as well as keep your leg in place.



7. Turn your SpikerBox on!
8. If you hear a popcorn sound, congratulations, you have just heard your first neuron firing! If you are not sure whether you are listening to spikes or noise, lightly touch spines located on the tibia with a toothpick. If you don't hear spikes in response to toothpick stimulations, try reinserting your electrodes, switching which one is in the coxa and femur. Once you are hearing spikes consistently, continue to the next section.

DISCUSSION QUESTIONS

1. When using a laptop, why do you need to unplug the computer and increase the screen brightness to maximum? What methods do researchers use to decrease electrical interference?

2. Why does lowering the temperature of the cockroach make it stop moving? What are other ways you could anaesthetize it?

3. Why do you place petroleum jelly on the removed leg and the cockroach following the surgical procedure? Explain.

4. Based upon your initial results, are you primarily listening to spikes coming from motor neurons (neurons that tell muscles to contract) or to sensory neurons (neurons that send information from the periphery into the brain). Why?

Exercise 2: How to Record and Analyze Spikes

In this exercise, you will use several methods to record data from your cockroach leg. Now that you have successfully witnessed spikes you are ready to record, quantify, and graphically present electrophysiology data.

Once your mesothoracic prep is up and running, listen to the spikes coming from the SpikerBox speaker. Can you understand what the neurons are saying to each other? What patterns can you detect while listening?

Procedure – Initial Setup

1. Plug the audio adapter cable into the SpikerBox and your computer. Note: for the SpikerBox to record properly, your computer's audio input jack must be audio only, not a combined audio In/Out jack.
2. Turn on Audacity, open a New window, and ensure the setup described in the previous exercise has been completed.
3. To record from your SpikerBox, simply click the Red Circle at the top of the screen. You can stop recording at any time by pressing the Yellow Square. If you begin to record again, the new "Track" will appear below the previous recordings. You can rename each track by selecting the Audio Track button next to your waveform. If you want to delete a track, select the X button in the upper left of each track.
4. Turn off your cell phone and Wi-Fi. Signal interference from these devices is significant. Ask your teacher permission to send a classmate a text. What does this tell you about how cell phones work?
5. During each experiment, it is wise to record without stopping. Noise created from cell-phones, moving the electrodes, or a variety of other sources can be removed prior to analysis. However, turning your recording on and off may become confusing. The easiest way to keep track of what your data corresponds to is to keep good notes in the space provided.
6. When you save, Audacity will save your "Project" in two forms that may be confusing. The first file saved is a folder ending in "_data" and the second is a file ending in ".aup." The .aup file must be in the folder holding the _data folder. In other words, keep the .aup file in the parent directory of the _data folder.

Procedure – Rate Coding

You can now begin experimenting with your cockroach leg! With your cockroach leg, you will compare how neurons in the leg communicate with no stimulation, a light touch from a toothpick, and a strong blow of air through a straw.

Think about how your brain can tell the difference between a finger lightly touching your arm and a more forceful poke. There are several reasons for why you differentiate the two stimuli. First, the light touch likely stimulates nerves from a very small area while the more forceful poke may stimulate neurons from much more of your arm. Second, the light touch may only stimulate neurons that respond to being compressed. These neurons, among them Merkel cells, send action potentials to the spinal cord and brain in response to light touch. These cells fire faster when stimulated. Therefore a light touch increases their firing rate, which in kind is interpreted by your spinal cord and brain as a light touch. With the more forceful poke, the Merkel cells fire faster, but they may do so much more strongly. Additionally, there may be other neurons stimulated for fire faster that convey another sensation such as pain.

1. Once your cockroach leg is prepared and Audacity is up and running, begin collecting data from your SpikerBox. Ensure your setup is functional by poking the leg a few times. If you see no response, you may need to adjust your electrodes.
2. When you have stable recordings, isolate your leg from any wind and begin the actual recording. Take notes when anything happens to the leg.
3. Record the spontaneous spiking patterns from the leg for 5 minutes. Note the beginning and end time of your control recordings in **Table 1**.
4. Take a toothpick and stimulate the hairs on the tibia of the leg. Try several variations of stimulation including constant pressure or repetitive poking, until you find one that gives you consistent reactions. Write your stimulation method in **Table 1**.
5. Once you have a stimulation method, stimulate the leg repeatedly for 5 minutes. If you need to take a break, do not stop your recording. Take note of the times you are stimulating or resting in **Table 1**.
6. Next, take a drinking straw and blow on the leg. How does the leg react to this stimulation? Try blowing with different amounts of force. Find an amount of force that allows you to maintain a relatively constant flow on the leg.
7. Blow on the cockroach leg for 5 minutes. As with the toothpick, take note of times you need to break, but do not stop your recordings. What happens to the reaction to your blowing over time and after breaks?

Table 1. Experimental Conditions

Condition	Method Notes	Timing Notes
No Stimulation		
Toothpick Stimulation		
Blowing Through Straw		

Basic Analysis of Spikes

Use the following method to isolate sections of your recordings.

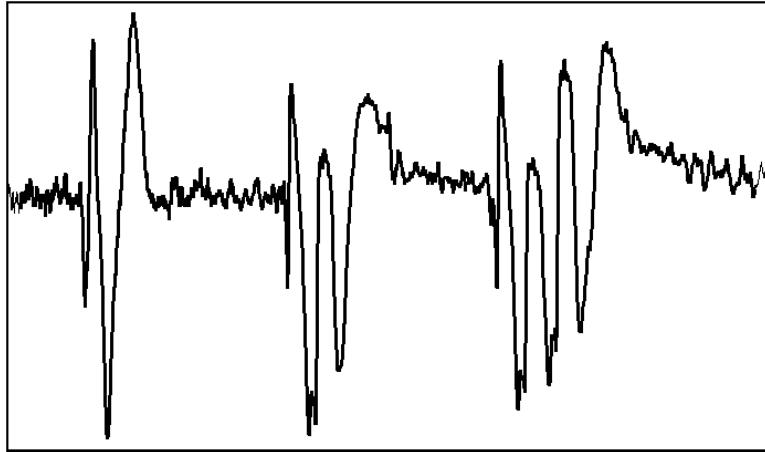
Find a representative section of spikes between 5-10 seconds long and select it with your cursor. Press the left click and drag across the waveform to select. Select a region with clear spikes and as little noise as possible.

Copy the selection (**Control-C**), open a new Audacity window (**Control-N**), and paste the selected 5-10 second waveform (**Control-V**).

In the **Effect** drop down menu, select **Amplify**. A dialogue box will appear which asks you to select the amount of amplification. The default selection is the amplification that keeps the audio from reaching beyond 1.0 or -1.0 on the scale. Record the **Amount of Amplification** value from the dialogue box in **Table 1**. Use the default settings and click **OK**.

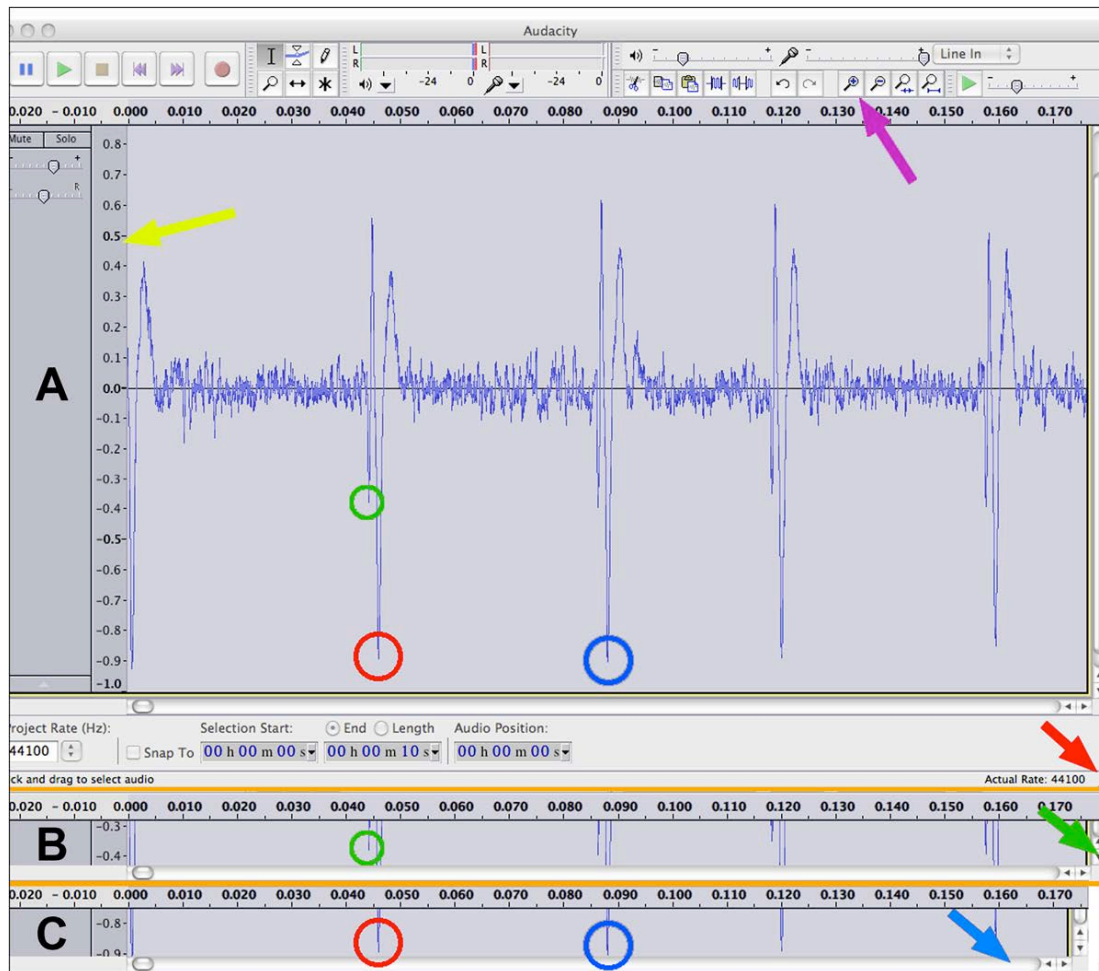
One of the first things you may see is that spikes do not always occur in a regular fashion.

You may also notice that not all spikes look the same. As shown in Figure 9, spikes may have 2, 3, or even 4 peaks to them. These are not special action potentials, but groups of action potentials.



Amplitude Analysis - Distribution of Action Potential Amplitudes

For this exercise, you will be asked to quantify the relative frequency and size of action potentials produced by a cockroach leg in response to your stimulations. Figure 10 provides a screen shot of Audacity that will help you in this process. You will be categorizing the spike peaks by amplitude in a process called **binning**. The bins will be the scale used by Audacity (Yellow Arrow). Keep in mind that you are measuring negative peaks.



Isolate and amplify a 5 second section of recording using the above method. Ensure you have recorded the **Amount of Amplification** in **Table 2**.

Widen your Audacity window to fill the width of your screen using the **Window Resize** tool (Red Arrow).

Use the **Zoom In** tool (Fig. 10A; Purple Arrow) so that you can visualize a small section of your trace, and easily identify the peaks of your spikes. At this point it is easy to eyeball the difference between peaks like those circled in Green and Red. However, for the purposes of quantification, you want to have a consistent

methodology that will allow you to reproducibly determine if the peak circled in Blue is different than that in Red.

Look over your data briefly and decide on a threshold for what is noise and you will accept as data. If a peak measuring between -0.2 and -0.3 is sufficiently distinct from the background noise, use this as your minimum negative threshold. Your threshold may be a larger negative number depending on how much noise was recorded. Write the **Threshold Value** in **Table 2**, keeping the number in tenths (i.e. -0.2, -0.3, -0.4).

Table 2. Amplification and Threshold

Condition	Amplification	Threshold
No Stimulation		
Toothpick Stimulation		
Blowing Through Straw		

Using the **Window Resize** tool (Red Arrow), shrink the Audacity window vertically, so that the scale on the left of the screen shows only 0.1 of the total waveform (Fig. 10B). This will allow you more accurately determine the bin a peak is in. Some peaks are clearly inside one bin, but some, like the one in the Blue Circle, require a judgment call on your part. Remain consistent in these close calls.

Record in **Table 3**, using hash marks, the number of peaks that end in the bin you have isolated. For example, the peak in the Green Circle has a minimum inside the -0.3 to -0.4 bin, while the peaks found in the Red and Blue Circles do not. Clicking the empty slider at the bottom of the window (Blue Arrow) will move the waveform smoothly, and will allow you to quickly count the peaks that fall within each bin. In many instances, you may have no peaks across an entire waveform inside a bin; this is OK.

Once you have counted all peaks in a bin for the entire 5 second waveform, click the down arrows (Green Arrow) to isolate a new bin. Record the number of peaks in each bin in Table 3.

Table 3. Spike Amplitudes – No Stimulation

Bin	Peaks Observed	Total
-0.2 to -0.3		
-0.3 to -0.4		
-0.4 to -0.5		
-0.5 to -0.6		
-0.6 to -0.7		
-0.7 to -0.8		
-0.8 to -0.9		
-0.9 to -1.0		

Table 4. Spike Amplitudes – Toothpick Stimulation

Bin	Peaks Observed	Total
-0.2 to -0.3		
-0.3 to -0.4		
-0.4 to -0.5		
-0.5 to -0.6		
-0.6 to -0.7		
-0.7 to -0.8		
-0.8 to -0.9		
-0.9 to -1.0		

Table 5. Spike Amplitudes – Blowing Through Straw

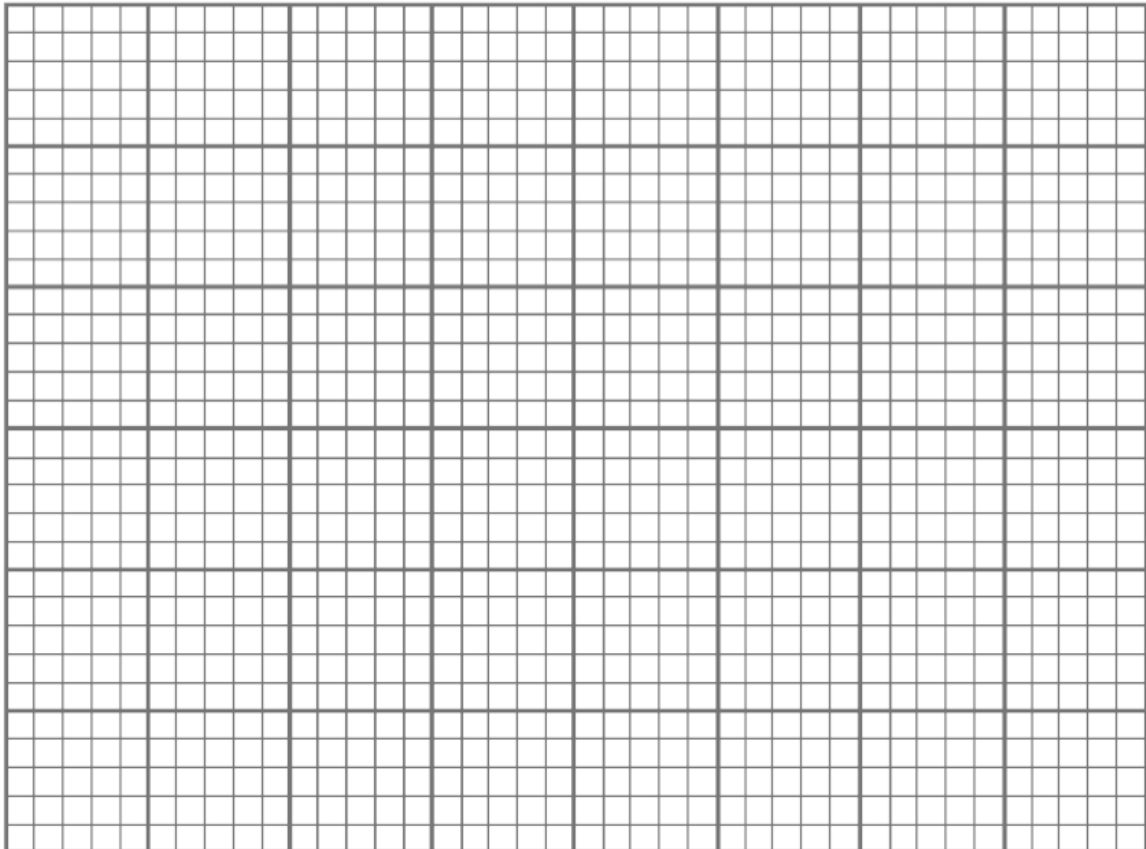
Bin	Peaks Observed	Total
-0.2 to -0.3		
-0.3 to -0.4		
-0.4 to -0.5		
-0.5 to -0.6		
-0.6 to -0.7		
-0.7 to -0.8		
-0.8 to -0.9		
-0.9 to -1.0		

Plot the total number of peaks from each bin on Graph 1.1 for each of your experimental conditions. Draw and label a line for each condition. Make sure you include units and label your axes.

Answer the following:

1. What is the independent variable (x-axis)? _____
2. What is the dependent variable (y-axis)? _____

Graph 1.1 Title: _____



DISCUSSION QUESTIONS

1. How do you compare the amplitude values of different peaks once the signal has been amplified?

2. How can you tell which electrode is your recording electrode and which is your ground?

3. How did the neurons respond to blowing? Was there any sort of attenuation in the neural response over time?

4. How many neurons were excited by the toothpick? How many by the straw? How do these responses differ from one another?

LAB THREE: EFFECT OF TEMPERATURE ON NEURONS

OVERVIEW

In this lab you will:

1. learn how changes in temperature affect neural spike firing
2. observe the temperature limits at which neurons cease firing

OBJECTIVES

Before doing this lab you should study:

- how action potentials are generated
- how sodium and potassium channels operate
- how temperature affects chemical reactions

After doing this lab you should be able to:

- explain the way that neurons produce action potentials
- describe why low temperatures would prevent a neuron from producing action potentials
- design an experiment to test for the optimal temperature range for neural firing

EQUIPMENT

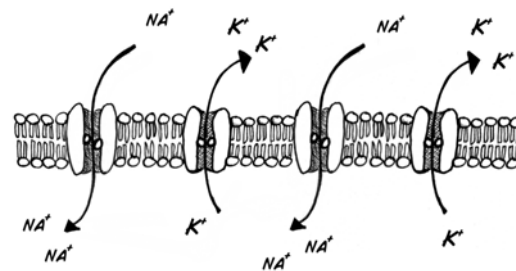
- SpikerBox
- Computer with Audacity installed or iPhone/iPad/Android device with Backyard Brains app
- Associated Laptop Cable or iPhone/Android Cable
- Cockroach
- Dry Ice or access to a freezer
- A candle
- A box approximately 5 – 6 inches taller than the candle
- Digital thermometer
- Dissection scissors
- Toothpick

INTRODUCTION

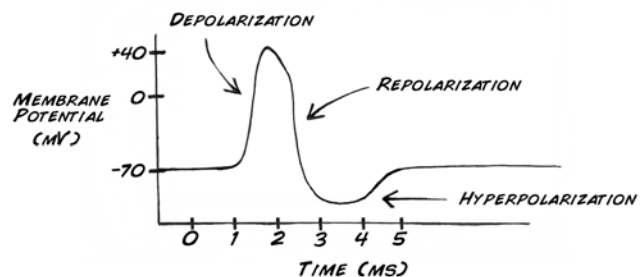
In order to understand the effect that temperature can have on neural communication, one must first understand some specifics about how that communication takes place. We discussed the basics about action potentials (APs) in Lab One; APs are generated at the cell membrane through the movements of various ions (Na^+ , K^+ , Cl^-) across the membrane. Let's look closer at the mechanisms of that process to see how temperature factors in.

The cell membrane is a double layer of lipids (i.e. fats) that are hydrophobic (they don't mix with water). This **lipid bi-layer** is the barrier between the inside of the cell and the outside of the cell, allowing the cell to maintain a different concentration of ions inside the cell than outside the cell. Thus, ions align on both the inner and outer surfaces of the membrane with an excess of positive charges (Na^+ ions) on the outside of the membrane and an excess of negative charges (Cl^- ions) on the inside. The charge separation creates a difference in electrical potential, or voltage, across the membrane called the **resting membrane potential**. The potential outside the cell is defined as zero, so thus the resting membrane potential is then negative, usually in the range of -60 mV to -70 mV.

How, then, do ions flow through the membrane to produce action potentials? The ion currents are carried into and out of the cells through **ion channels**, proteins that span the cell membrane and can open to conduct specific ions across to the other side. In nerve cells, ion channels conduct ions at extremely high rates (up to 100 million ions per second). Ion channels can be active at rest, or activated due to an external stimulus (such as voltage or chemicals). Resting channels in cell membranes can be selective for Na^+ , K^+ , or Cl^- ions, and it is in fact the resultant concentration of ions inside and outside of the cell due to these channels that are most responsible for the resting membrane potential. Ion pumps also actively move ions into and out of the cell to maintain that stable membrane potential at rest.



During an action potential, Na^+ channels are activated by changes in voltage that allow the Na^+ ions layered on the outer cell membrane to rapidly diffuse into the cell and thus push the inner membrane potential highly positive (depolarization). This positive membrane potential causes the Na^+ channels to close and K^+ channels to instantly open, allowing the K^+ ions inside the cell to rapidly diffuse out of the cell and drop the membrane potential sharply in the other direction (repolarization). At this point the K^+ channels close, but the K^+ outflow current has brought the membrane potential below the resting state (hyperpolarization). With the voltage-gated ion



channels closed, the resting channels again dominate and a slower influx of K^+ ions returns the cell membrane to its resting state.

Recent research has demonstrated ion channel activation has a dependence on temperature. Collins et al 1972 demonstrated that the conductance of Na^+ channels is dependent upon temperature with an Arrhenius temperature relationship (i.e. an equation to relate the rate constant of chemical reactions to temperature) defined by this equation:

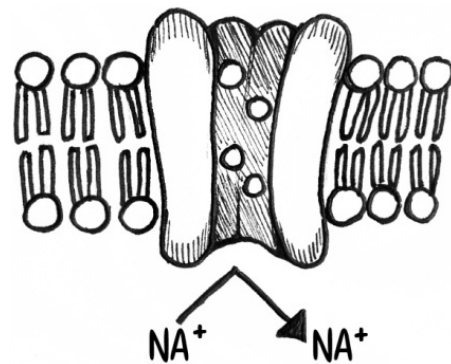
$$\ln(1/T) = \ln F - E_a/(RT)$$

Where T is temperature, E_a is the difference in energy level between an activated ion channel and a non-active state, and both F and R are constants. If we solve that equation for the activation energy,

$$E_a = RT \cdot \ln(TF)$$

We see that activation energy increases as T increases, and conversely that it would lower as T decreases. The take-home for our purposes is that the ion channel is more active at higher temperatures (more likely to be open), and less active at lower temperatures. Fohlmeister et al demonstrated in 2009 that sodium channels in retinal ganglion cells were consistently “sleepy” for temperatures below 8 degrees Celsius, with a loss of spiking for $T < 7^\circ C$ (note: different cell types have different temperature ranges, and the neurons in this experiment can spike well below $7^\circ C$).

The next question is, why? Why is there such a temperature dependence for neural cell behavior? Wouldn't it be great if neurons worked just as well at all temperatures? The short answer is that every active cellular process, including the opening and closing of ion channels, is some type of chemical reaction that requires energy. As a general rule of thumb (though there are many exceptions) increasing temperature speeds up and increases chemical reactions, while decreasing the temperature slows them down. At extreme cold temperatures chemical reactions can stop happening altogether, which in this example could lead to ion channels not opening and thus no action potential initiation.



While increasing temperatures can speed up chemical reactions within certain ranges, we also have to consider that if temperature is increased too much this could damage the cell in other ways. The cell membrane is composed of lipids, and lipids transition from solid and ordered (called a gel) to liquid and disordered (called ‘fluid’) based upon temperature. These temperature transition thresholds are different based on the type of lipid and the



Lipid bilayer breaks down at high temperatures

environment, but once these thresholds are reached the lipid cell membrane can break apart which effectively results in the death of that cell.

SETUP

Computer Setup

Use of a computer for the basic aspects of this experiment is not required, as the SpikerBox is equipped with a small speaker. However, in order to perform some of the analysis described in this manual, a computer will be necessary.

There are several free options for capturing and analyzing data from your SpikerBox.

Backyard Brains iPhone/iPad App: Backyard Brains iPhone/iPad app, which can be downloaded via iTunes. This app can be found at the following link:

[Backyard Brains iPhone/iPad app](#)

To use this app, you will need to purchase or make an audio cable to link the SpikerBox to the iPhone.

Audacity: Audacity is a freeware program designed for editing and recording sounds. While not the original purpose of the software, Audacity is a perfect way to convert the amplified signal of the SpikerBox into visual and recordable data. Audacity runs on both Windows, Mac OS X, and Linux/Unix. The website for Audacity can be found at the following link:

[Audacity Homepage](#)

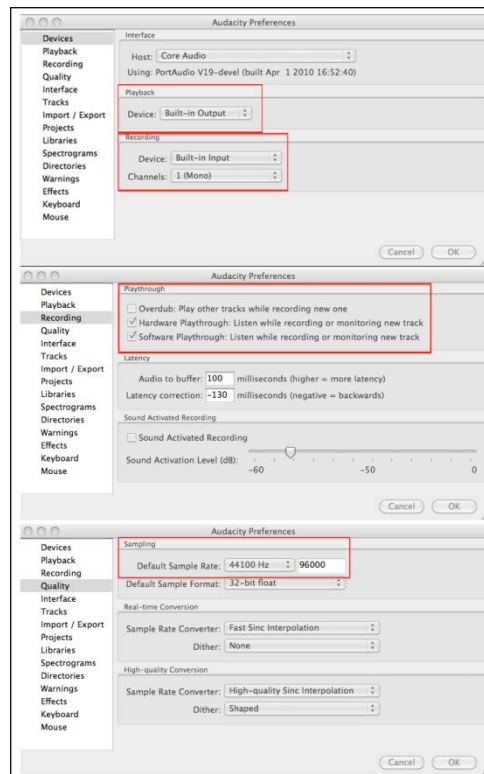
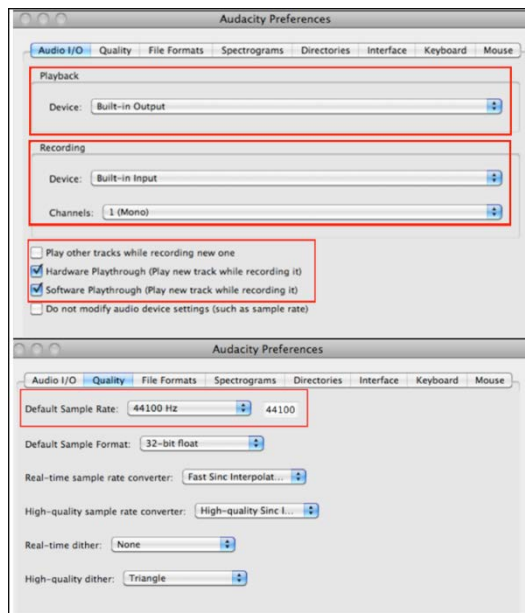
Once Audacity has been installed, refer to the following procedure:

Audacity Setup

1. If using a laptop, fully charge your battery and record from the SpikerBox while unplugged. When plugged in, the SpikerBox will amplify the current running through the electrical system of the building. The result will be an increase in electrical noise recorded by Audacity. Running off of battery power will decrease the amount of electrical noise.
2. If using a laptop, set the brightness of your screen to maximum. Similar to the affects of the surrounding electrical wiring on your recordings, a dimly lit LCD display will create a significant amount of noise that can be amplified by the SpikerBox.
3. Plug your SpikerBox into the Laptop with male-to-male audio cable. This is exactly the same as a headphone cable, except it has “male” connector on each end.
4. Open Audacity, go to **File → Preferences**. Note: Due to differences in Audacity between versions, there may be differences in how the **Preferences** window is organized. The primary settings you need to set up are the same, even if they look a little different on your screen.

5. Select the **Audio I/O** tab in the **Preferences** window. Some versions place this window in the **Devices** tab.
6. Select the **Built-in Input** as the **Recording Device** from the drop down menu.
7. Select **1 (Mono)** from the **Channels** drop down menu.
8. Select **Built-in Output** as the **Playback Device** from the drop down menu.
9. The following steps are found either in the **Audio I/O** or **Recording** tabs in the **Preferences** window. The following checkboxes are listed in order as they appear:
 - **Uncheck** the box: Play other tracks while recording a new one.
 - **Check** the Box: Hardware Playthrough
 - **Check** the box: Software Playthrough
10. Select the tab labeled **Quality**. Set the **Default Sample Rate** to 44100 Hz (or 44,100 times per second).
11. Audacity should now be configured to record from your SpikerBox.

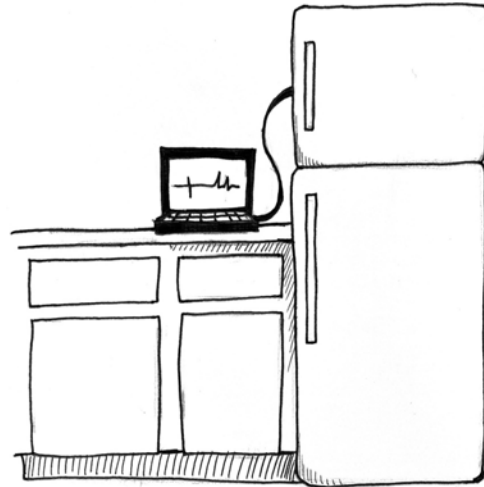
Below are examples of how your **Preferences** window should be configured. Keep in mind that your version of Audacity may look different, but the settings will be the same. Two examples are provided, with the important settings highlighted.



PROCEDURE

Exercise 1: Neural Response to Cold

1. Prepare a cockroach leg as described in Lab 1.
2. Place your SpikerBox in the freezer of a household refrigerator. Make sure that the cable is long enough to go from your laptop into the freezer, and stays connected to the SpikerBox you placed inside. Note that actively refrigerators can be very electrically noisy, so you may want to unplug the cold freezer or, as an alternative, use a cooler of dry ice.
3. Place a digital thermometer inside the freezer
4. Listen to the neurons firing for 1 – 3 minutes
5. At a certain temperature the spikes should go away. Open the freezer and record the temperature displayed on the thermometer. Hint, the temperature should be below “freezing”, as typically 31° - 33° F is not cold enough. If you use water ice instead of dry ice in your cooler, your spikes will not go away.
6. Remove your SpikerBox and digital thermometer from the freezer, and listen until the neurons start firing again. Make note of the temperature, and how long it takes before the spikes come back.
7. Repeat steps 1 – 6, three times



COLD LIMIT

RECOVERY
TEMPERATURE

LEG 1

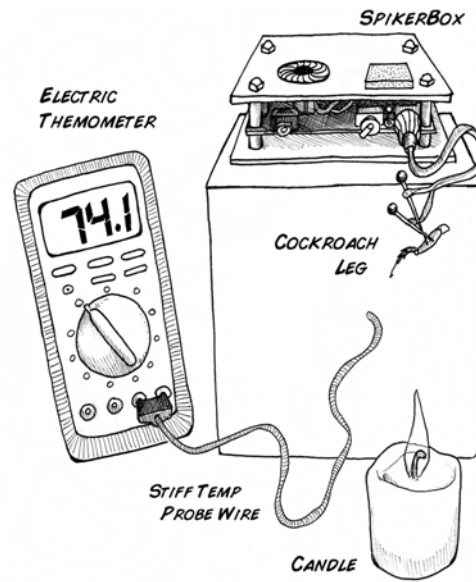
LEG 2

LEG 3

AVERAGE

Exercise 2: Neural Response to Heat

1. Place the SpikerBox on top of the box, and have the electrode with the cockroach leg attached hanging out "in space" 5 – 6 inches over the top of the candle
2. Position the wire of the digital thermometer such that you can measure the precise temperature where the leg is. Record the initial temperature in the room.
3. Light the candle
4. Listen to the neurons firing. As the temperature slowly rises, you should hear the neurons responding
5. At a certain temperature, you will hear the neurons discharge with a sound like a very low scream of spikes. Record the temperature at which this happens, and put out the candle
6. When the thermometer indicates that the leg is back at the initial room temperature, test to see if the spikes come back
7. Repeat steps 1 – 6, three times



HOT LIMIT

RECOVERY TEMPERATURE

LEG 1

LEG 2

LEG 3

AVERAGE

DISCUSSION QUESTIONS

1. How did the neurons respond to being placed in low temperatures? Was the response sudden, or gradual? Explain.

2. On very cold winter days (in some parts of the world) the outside temperature can go below the temperatures used in this experiment, yet animals can still survive and move. What are some possible reasons for why the neurons in these animals don't stop firing?

3. How did the neurons respond to being placed in very high temperatures? Was the response sudden, or gradual? Explain.

4. At both temperature extremes the neuron stops firing, but for one of the extremes the cell resumes firing when returned to room temperature while for the other it doesn't. Which is which, and what might this difference reflect?

Experiment FOUR: Somatotopy

OVERVIEW

In this lab you will:

1. learn about the way that the nervous system organizes somatotopic input from peripheral sensory structures;
2. observe the selectivity of neural responses based upon the specifics of the recording locations; and
3. investigate some basic principles on the somatotopic organization of the cockroach leg, nerves and ganglia.

OBJECTIVES

Before doing this lab you should:

- study how output sensory structures, peripheral nerves, and the spinal cord convey sensory information to the brain
- study the primary somatosensory cortex and how the brain uses these structures to interact with the outside world
- note analogies between the neural mechanisms for sensation and motor function (i.e. finger, nerve, spinal cord, brain, back down) and more simple mechanical systems such as an alarm (i.e. laser beam, wire, relay, computer, chime)

After doing this lab you should be able to:

- explain why manipulating one sensory barb on the cockroach leads to a different neural response than manipulating at a different location
- design an experiment to map out which central nervous system ganglia convey information from which sensory barbs on a cockroach leg
- describe what a somatotopic arrangement means to the study of sensory deficit pathologies (i.e. blindness, deafness, paralysis, etc.)

EQUIPMENT

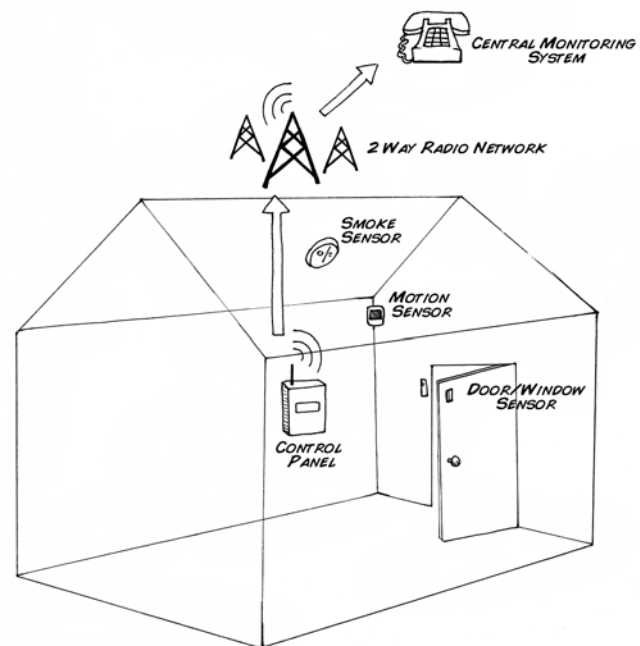
- SpikerBox
- Computer with Audacity installed or iPhone/iPad/Android with Backyard Brains app
- Associated laptop cable or iPhone/Android cable
- Cockroach
- Dissection scissors
- Toothpick

INTRODUCTION

The human brain recognizes sensory cues and initiates complicated sets of motor behaviors in ways that no current computer can come close to replicating, making every sight or touch that we experience and every voluntary action we take a masterpiece of engineering. The “touch” system in people is governed by the **somatosensory system** and, much like human-engineered devices, it is the hardware and wiring comprising the system that allows us to perceive pressure. Thus, understanding the organs and connections that comprise the somatosensory system is the key to understanding how we interface with the world.

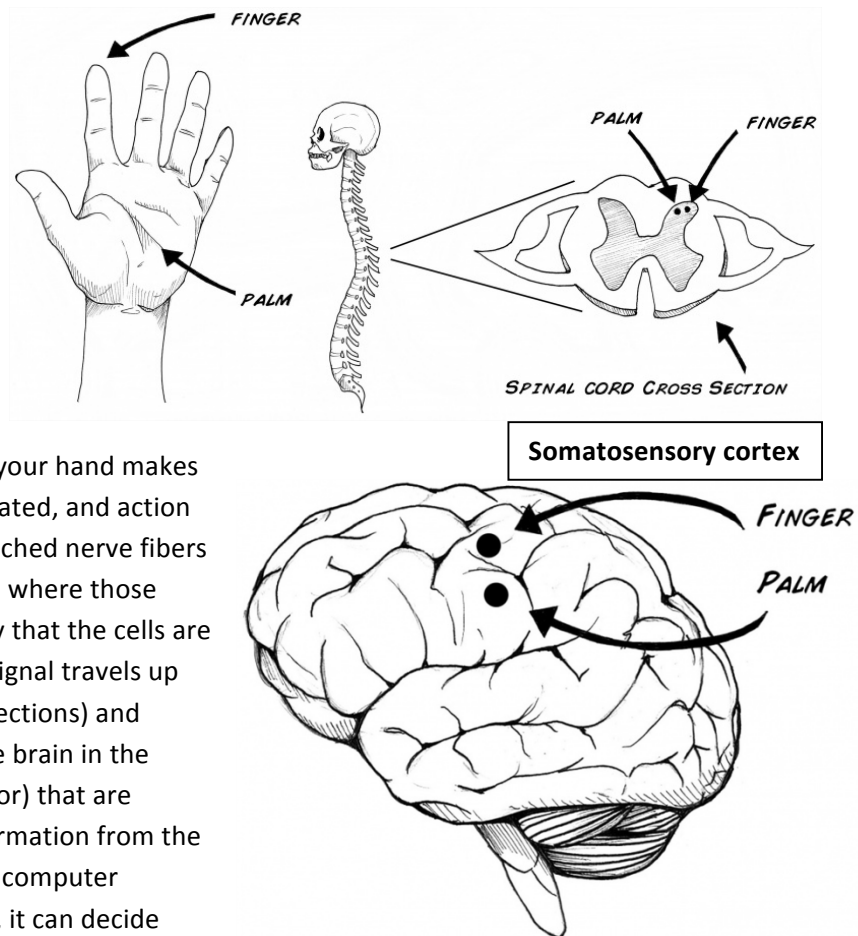
Before we move on to the more complicated and intricate biological somatosensory system, let's first look at a simpler example of human engineering: the alarm. A home alarm system might have multiple ways to detect an intruder. Contact sensors on the doors or windows maintain a closed circuit for as long as the two sensor plates are connected, but when someone opens the door the circuit is broken, that signal is relayed and travels down a connection to a central decision-making location. Similarly, motion detectors can take advantage of simple radar, sonar, or laser beams to detect when a moving entity is in the room. More complicated systems might be able to detect changes in room temperature due to body heat, or sounds made in what should be quiet areas. But all of these ways to “sense” the presence of a person require some type of external sensing device that, when

activated, communicates its findings to some sort of relay that has a Boolean “on” (something is detected) or “off” (nothing has changed) setting. When the relay is activated, it reports its findings through a wire or (these days) wireless connection to some sort of computer processor that then decides how to react (e.g. warning chime, full alarm, place call to the police, etc.) based upon its programmed parameters.



Your somatosensory system works like a home security system.

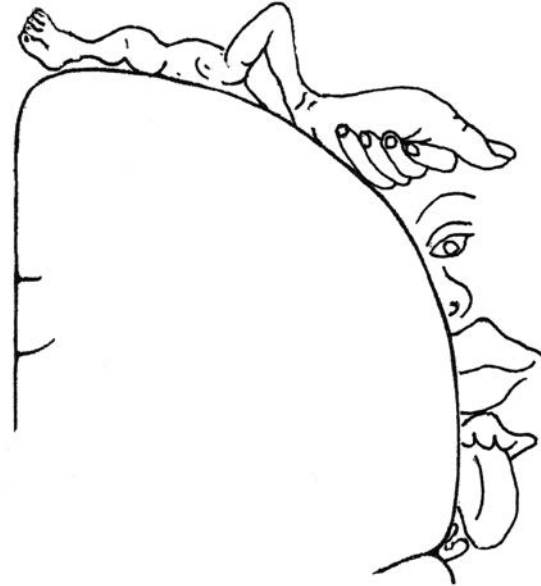
This process is very analogous to what goes on in a human sensing situation. Take, for example, the neural mechanisms responsible for the physical sensations one experiences when shaking hands. There are touch receptors in your fingers and palm that act as the sensing devices (the contact sensor, from the alarm example). When your hand makes contact, those receptors are activated, and action potentials are fired down the attached nerve fibers to specific parts of the spinal cord where those nerves are synapsed (i.e. the relay that the cells are hard-wired to). From there, the signal travels up the spinal cord (more wired connections) and synapses with specific parts of the brain in the neocortex (the computer processor) that are specifically attuned to tactile information from the fingers and palm. Once the brain computer processes this tactile information, it can decide how to react (e.g. maintain grip, tighten it, let go, etc.) based upon if you like the person or want to impress them.



You have learned about action potentials and synapses in Labs One, Two and Three, so you should already be familiar with the way that the signal travels along the nerves and spinal cord that connect the sensory devices to the brain. Thus, the key new information here is the way that these sensory pathways are hard wired through the spinal cord and the brain. The sensory neurons that convey information from the skin, muscles and joints have their cell bodies (i.e. the nuclei, the “brains” of the cells) clustered together in the **dorsal root ganglia** (DRG). The DRG are ball-shaped neural masses comprised of thousands of cell bodies, and are found directly next to the spinal cord along its length, inside the vertebral column. From the DRG, the sensory cells synapse directly onto the spinal cord. The spinal cord is made up of **gray matter** (more cell bodies) and **white matter** (fibers); the gray matter containing sensory nuclei is grouped into what is called the **dorsal horn**, while the white matter surrounding it is divided into columns containing bundles of axons that carry the sensory information up the spinal cord.

The central axons of the dorsal root ganglion cells form a neural “map” of the body surface when they terminate onto the spinal cord. This ordered arrangement of inputs from different parts of the body surface, called **somatotopy**, is maintained through the entire somatosensory pathway up to the brain.

The sensory information enters the brain in a region called the **thalamus**, essentially the gatekeeper for information to the cerebral cortex. Axons from the thalamus project to the neocortex and terminate in the **primary somatosensory cortex**. All portions of the body are represented in the cortex, in proportion to how many sensory nerves cover an area. A to-scale map for how much cortex area is dedicated to each body region is called a **homunculus**. There are more sensory nerves in the face, for example, than in the body trunk...so even though the trunk has a much larger physical mass than the face, the face portion of the somatosensory cortex is larger than that of the trunk portion.



What we've described here just hits the tip of the iceberg for how somatic information is processed in a person. In the body every connection between the peripheral touch receptors and the brain performs a bit of information processing,

Sensory homunculus Map by Dr. Walter Penfield (Wikipedia.org)

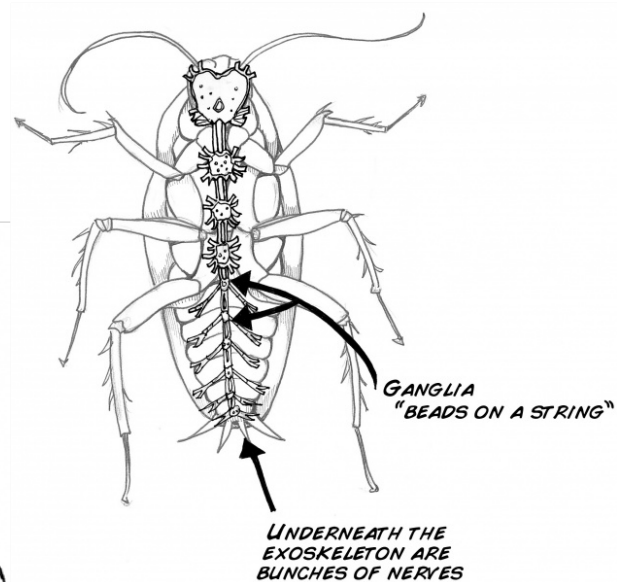
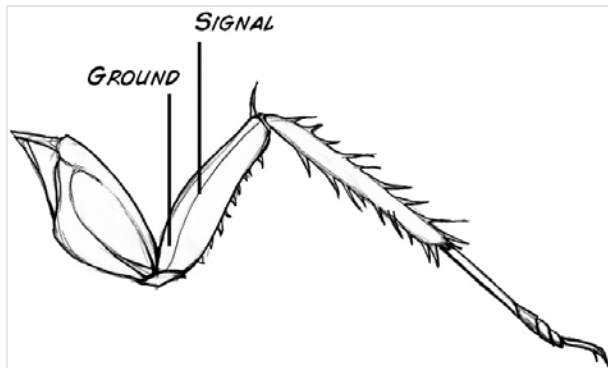
modifying the way that the info is packaged such that it increases the computational capacity of the brain. Also, each type of somatic sensation (e.g. touch, pain, position) is processed through different pathways that end in different brain regions. And sometimes the response to a sensation is hard-wired in such a way that the body responds before the signal even reaches the brain. Think of how quickly your body reacts to a painful sensation, such as putting your hand on a hot stove...your hand jerks backwards before you even necessarily realize what you've done, by reflex. These reflexes are governed at the level of the spinal cord to ensure quicker reactions without having to wait for the brain to decide anything.

But we can explore that type of complexity in another lab, on another day. The cockroach sensory system is actually a bit simpler than the human version described, with fewer nerves and connections. In the cockroach, the sensory barbs on the legs connect with the ganglia found along the midline of the cockroach (analogous to the human spinal cord). For today, let's use our cockroach models to take a closer look at some basic relationships between sensory receptors and the internal information map that they provide to the organism.

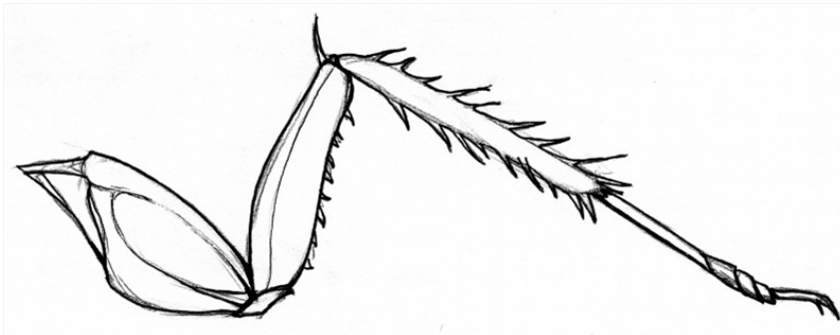
PROCEDURE

Exercise 1: Record responses to stimulating motion-sensitive barbs

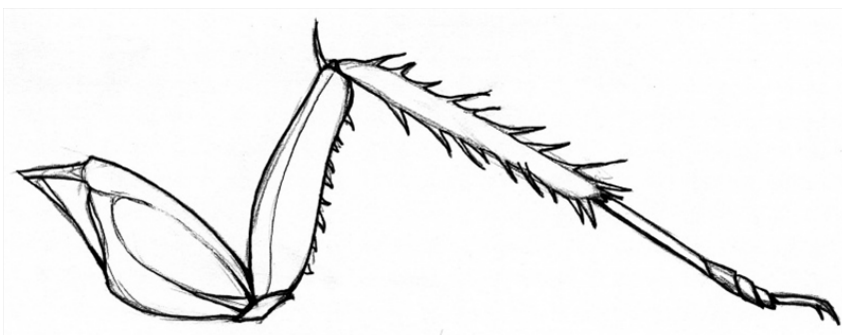
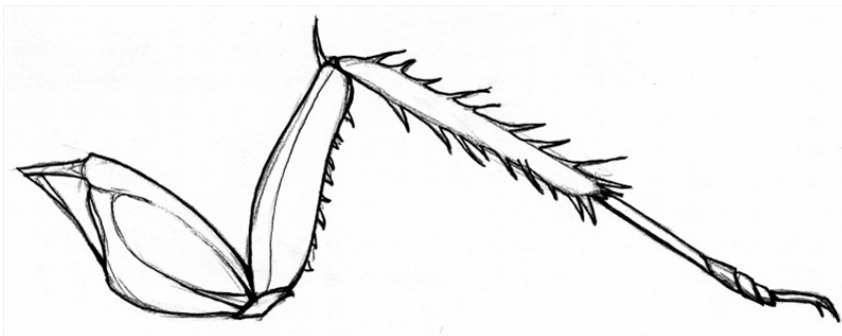
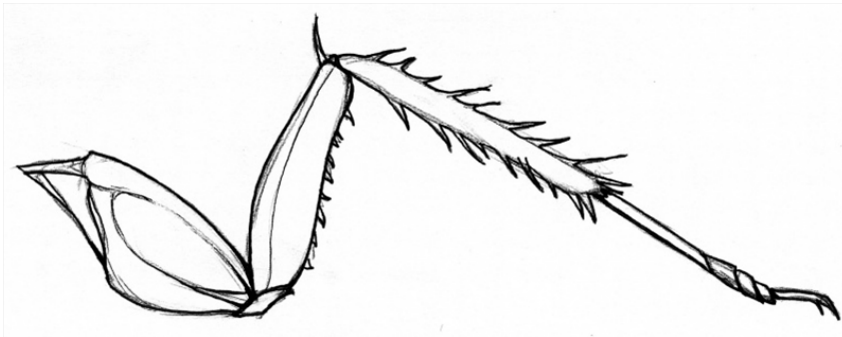
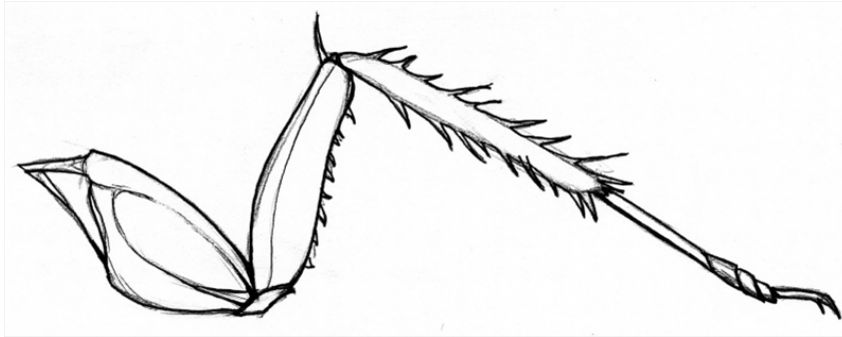
1. Plug in your smartphone or set up your computer with Audacity as previously described in experiment 1, and prepare a cockroach leg as also described in experiment 1.
2. Place both the ground and signal electrodes into the femur, as shown



3. The leg is covered with about 20 barbs that are sensitive to motion. Using a fine-tipped toothpick, try to touch each barb as you are listening to the spikes.
4. Try to find a barb that, when touched with a toothpick, causes vigorous changes in the spiking activity. Circle the responsive barb on the diagram and draw the general location of your recording electrode as well. Note the general firing characteristics (firing frequency, relative auditory volume of spikes, etc.) of the responsive barb in your notes.
5. Repeat for different legs on your cockroach, and for multiple cockroaches.

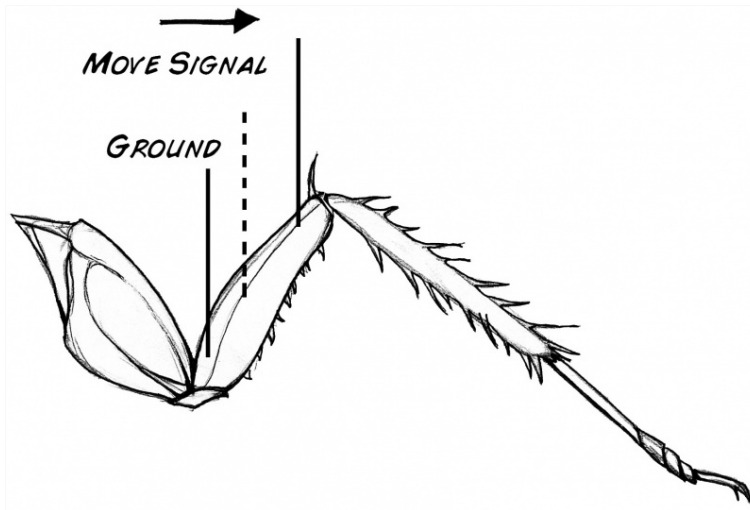


**Extra diagrams of cockroach legs to mark the activating barb and electrode location.
Make copies as needed.**

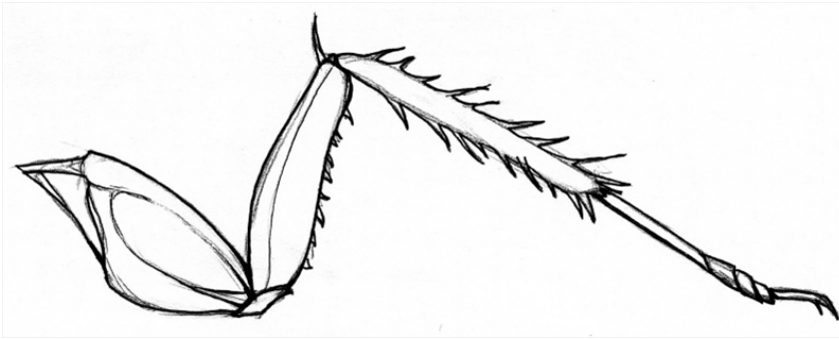


Exercise 2: Move recording electrode and re-map

1. Move the recording electrode to a different place in the femur.



2. Repeat steps (3) and (4) from Exercise 1 to identify a responsive barb, if possible.



3. Repeat at least 3 times within a given leg, and repeat the entirety of both exercises on at least 3 different legs. Note: you might observe different firing characteristics based on placement.
4. From your notes and diagrams, try to create a general "map" of the relationship between which barb was responsive and the location of your recording electrode.

DISCUSSION QUESTIONS

1. Did you observe differences in the barb/recording location relationship from one leg to another on the same cockroach? How about legs from different cockroaches? What do your answers suggest about peripheral nerve mapping in the cockroach?

2. Did the quality of your neural responses change in a negative way as you moved the recording electrode around within a single leg? If so, what might be causing the signal degradation? Do you think this will affect your observation of somatotopy?

3. If instead of recording from the femur, you instead recorded further “up the path” of the nervous system in the cockroach ganglia, would you expect the cell firing patterns to be the same or different? Why?

FUTURE EXPERIMENTS

The third discussion question actually makes a good foundation for future experiments: repeat this exact experiment, but with the recording electrode in one of the ganglia in the body of the cockroach as opposed to in the tibia. If you are able to find a ganglion and record neural information, test how the neural firing changes as you move from one barb to another. At the end, compare the behavior of different ganglia with the behavior of the tibial nerves.

LAB FIVE: MICROSTIMULATION

OVERVIEW

In this lab you will:

1. learn how to prepare a cockroach leg for microstimulation;
2. test how different stimulations cause spikes in motor neurons;
3. observe the difference between sensory and motor spiking patterns; and
4. investigate how different types of stimulations affect the generation of action potentials in a cockroach leg preparation.

OBJECTIVES

Before doing this lab you should understand:

- how neurons communicate with muscle cells
- how electrical stimulation can cause a muscle contraction

After doing this lab you should be able to:

- explain how a nervous system controls muscle movement
- describe how distinct movements are caused by specific spiking patterns
- design an experiment to map ideal frequencies for stimulation

EQUIPMENT

- SpikerBox with audio cable adapter
- computer with Audacity installed
- cockroach
- ice water or access to a freezer
- dissection scissors
- toothpick
- microstimulation electrodes
- electrical signal generator (iPhone or MP3 Player)
- Y-splitter (optional)
- external speaker (optional)

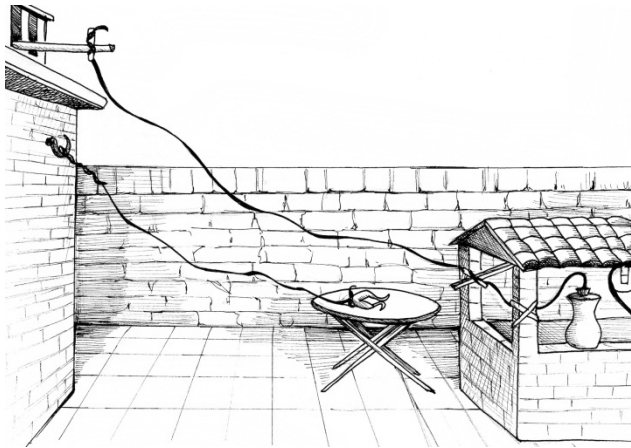
INTRODUCTION

Long before scientists were able to record spikes, they to stimulate the nervous system using batteries (Leyden Since nerves use electricity to communicate, they can be manipulated with electricity as well. Luigi Galvani, an scientist in the 1700's, discovered that electricity applied to of frog legs caused the large muscles to twitch.



were able
Jars).

Italian
the nerves



Such discoveries led to debates at the time as to whether “animal electricity” was different from the electricity during lightning storms. Galvani also tested this by hanging frog legs off his back porch during thunderstorms & watching the legs twitch. These results were also inspiration for Mary Shelley’s “Frankenstein.”

“Perhaps a corpse would be re-animated; galvanism had given token of such things: perhaps the component parts of a creature might be manufactured, brought together, and endured with vital warmth.”

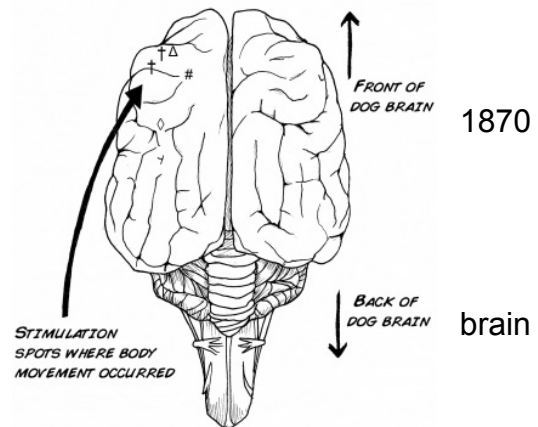
-Mary Shelley, Introduction to Frankenstein

Eventually the scientific community agreed and discovered that while electricity can indeed stimulate nervous system and muscle tissue, the tissue itself generates electricity. This led to the beginnings of contemporary neuroscience, which you are studying today.

We learned about action potentials (APs) and neuron-neuron synapses in Lab One, but the galvanic muscle responses are a result of synapses between motor neurons (MNs) and a skeletal muscle, or neuromuscular junctions. Unlike the convergent connections on central nerve cells, a postsynaptic muscle cell is normally innervated by just a single presynaptic MN at a specialized region of the muscle membrane called the end-plate. Acetylcholine (ACh) is released by the axon terminal from the MN, which directly opens voltage-gated Ca^{+2} ion channels in the muscle membrane that allows Ca^{+2} to enter the

terminal with each action potential. Motor neurons excite the muscle by opening ion channels at the end-plate, producing a large amplitude end-plate potential that rapidly activates voltage-gated Na^+ channels and produces an action potential that propagates along the muscle fiber and generates movement.

In another famous experiment, German medical scientists Eduard Hitzig and Gustav Fritsch in applied electric current to the exposed cerebral cortex (wrinkly part of brain) in dogs in their kitchens (yes, it was odd even back then) showing that stimulation of different parts of the brain can cause different types of movements.



Similarly, it has been shown that stimulation of the nerve cord can produce rhythmic patterned outputs to leg muscles, termed central pattern generators (CPGs). T. Graham Brown demonstrated as early as 1911 that these coordinated spiking patterns can induce the basic muscle responses of stepping without the need of descending commands from the cortex.



Today, such central-stimulation/motor-sensory-output techniques are used in patients, most notably those afflicted with Parkinson's disease. By inserting a small, long electrode into a specific part of the brain called the subthalamic nucleus, the shaking and tremors associated with the disease can be lessened. Sometimes there are side-effects though, like increased gambling & other compulsive behaviors.

Today, some advanced research groups are designing small chips that stimulate the nerves of the eye as a cure for blindness.



SETUP

Microstimulation Electrode

The microstimulation electrode will act as a conduit between your electrical signal generator and the cockroach. The electrode will consist of an audio cable that plugs into your signal generator as a means to deliver the stimulation to the cockroach. You can choose to solder either pins to your audio cable, similar to those used in Lab 1, or use alligator clips that can be attached to the electrodes that come with your SpikerBox.

Electrical Signal Generator (ESG)

There are many programs that can generate electrical stimuli ideal for this lab. If you are using an iPhone or iPad, these free apps can be found at the iTunes store at the following links:

[AudioSigGen](#)
[FreqGen](#)

If you are using a PC, you can use this online software:

[Rhintech](#)

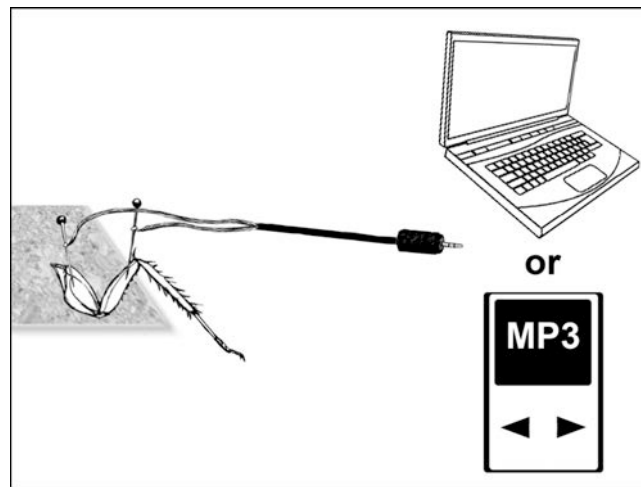
Additionally, you can simply download various frequencies as MP3s and play them through any MP3 player. Here is a website from which you can download free frequencies appropriate for this exercise:

[TestSounds](#)

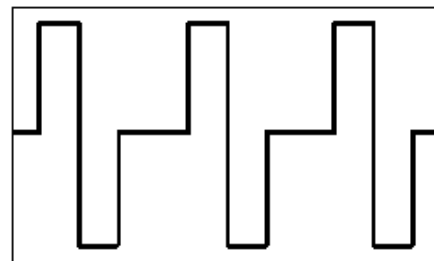
PROCEDURE

Exercise 1: Cockroach Leg Microstimulation Preparation

1. Prepare a cockroach leg as described in Lab 1.
2. Attach the microstimulation electrode. If using the alligator clip electrode, attach the clips to the SpikerBox recording electrodes inserted into the coxa and femur. If using a direct electrode, place the electrodes into the coxa and femur of your cockroach leg.
3. Plug the Microstimulation Electrode into an ESG such as a computer or MP3 player.



4. If you are able to, program your ESG to square waves.
5. Set your ESG to 50% volume (amplitude) generate a signal with a frequency of 200 repetitions per second).
6. Play the tone and observe the cockroach leg. If the leg moves in response to the stimulation, continue on to Exercise 2. If the leg does not move, continue to Step 7.
7. If the leg does not move in response to the stimulus, ensure you have your electrode plugged into the audio out jack and that the tone is being produced. You should be able to hear a 200 Hz tone at half volume with **EXTERNAL SPEAKERS**. If you do not hear a tone, you may need to adjust your audio settings.
8. If you can produce a tone with your ESG, then ensure that your cockroach leg is alive and you have your electrodes in the proper place. Using the protocol in Lab 1: Exercise 1, use your SpikerBox electrodes and speaker to ensure spikes are being produced by the leg. Ensure you have placed your electrodes in the coxa and femur.



produce
and
Hz (200

9. Ensure the soldering on your electrodes is intact and that your electrodes are not touching each other. Consult with your teacher if you continue to have problems stimulating your cockroach leg.

Exercise 2: Response of Cockroach MNs to Amplitude and Frequency

Now that you have a functioning Microstimulation Apparatus, it is time to measure the frequency and amplitude response properties for the cockroach motor neurons (MNs). This will be achieved by simple observation of your leg while stimulating it with an array of different tones.

1. Begin stimulation of the leg at the lowest frequency (20 Hz) and volume (lowest amplitude your device is capable of) shown in Table 1. Keep in mind, if you were to try to listen to the output of your ESG, you may not be able to hear a tone of 20 Hz no matter how loud you make it. Human hearing ranges between 20 Hz and 20,000 Hz (20 kHz), although there is variation between individuals.

CAUTION: DO NOT USE HEADPHONES FOR THIS EXPERIMENT IF THE VOLUME OF YOUR ESG IS ABOVE 50% AS YOU MAY CAUSE DAMAGE TO YOUR HEARING. ALWAYS USE AN EXTERNAL SPEAKER.

2. Using Table 1 as a guide, test the responsiveness of your leg to all amplitudes of each frequency. Mark each trial with either a (+) or (-) to signify whether the leg moved in response to the stimulation. If your ESG does not produce the frequencies listed in Table 1, use whatever frequencies you have and adjust Table 1 accordingly.

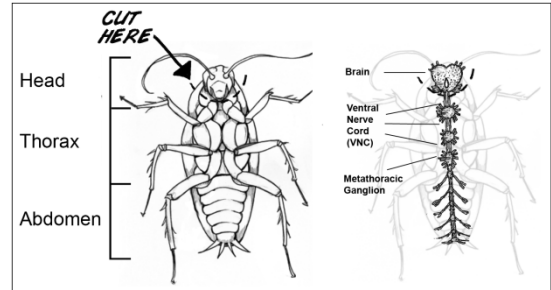
Table 1. Response by Frequency and Amplitude

Frequency	Min Output	$\frac{1}{4}$ Output	$\frac{1}{2}$ Output	$\frac{3}{4}$ Output	Max Output
20 Hz					
40 Hz					
80 Hz					
150 Hz					
200 Hz					
1000 Hz					
2000 Hz					
3000 Hz					
5000 Hz					
10,000 Hz					

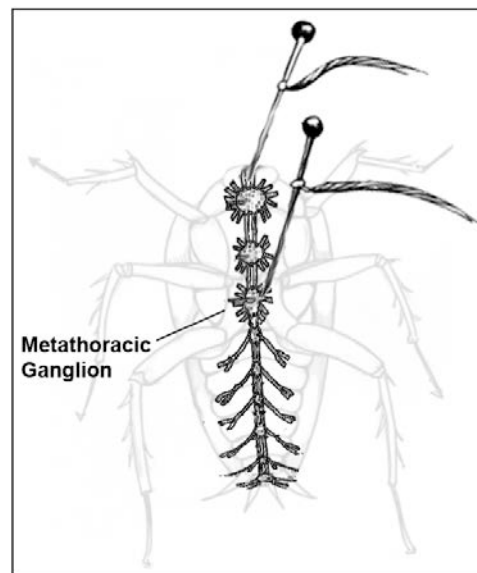
Exercise 3A: Recording and Spike Patterns from Cockroach Ganglia

Note: This experiment requires a cockroach to be sacrificed

1. Place a cockroach in ice water or a freezer. When the cockroach has been anaesthetized, place it on your lab bench.
2. Using your dissection scissors, decapitate the cockroach at the junction between the head and the thorax. Place some petroleum jelly on the cut.



3. Insert your SpikerBox recording electrode through the thorax of the cockroach into the **metathoracic ganglion**. Try to place the electrode slightly to one side of the ganglion. Stick the ground electrode into the thorax where the head used to be.




4. Plug your SpikerBox into your computer, turn it on, and start Audacity. **Begin recording** from the metathoracic ganglion. **Adjust the Y-axis** of the recording so that the spikes observed are maximized. **Adjust the X-axis** (using the magnifying glass tool in the menu bar) so that you can differentiate bursts directly related to movements.
5. For several minutes, observe recordings from the cockroach and take note of how they correspond to movements of the metathoracic legs. As the connections between the ventral nerve cord (VNC) and the metathoracic legs are intact, action

potentials are still able to propagate down motor neurons to the muscles. You should also be able to observe spontaneous movements from the cockroach. Keep in mind that you are likely to be observing three types of information while recording from the ganglion: a) motor information being sent from the ganglion to the leg muscles; b) sensory information being sent in to the VNC from the legs; and c) information being integrated and processed between ganglia along the VNC. Your goal is to identify spiking patterns that lead to motor movements.

6. Once you feel like you can see a relationship between specific leg movements and specific patterns of spikes, mark down the following pieces of data in the Table 2 for five different events: a) the time of the spikes; b) a description of the movement.
7. Stop recording from Audacity and find the spiking patterns that you noted in Table 2 in the trace. Highlight with your mouse and copy (Control-C) the small section of one spike series. Open a new Audacity file (Control-N) and paste the copied spike segment (Control-V). Do this for each of the 5 spike patterns from Table 2 so that you now have 5 new Audacity windows open.
8. For each spike pattern, amplify the trace under **Effect-Amplify** in the menu bar. Briefly sketch each pattern in Table 2. Save each spike pattern for later use.

Table 2. Spike Pattern Database

Spike Pattern	Time Recorded	Movement Description	Trace Sketch
Example	1:05.08 – 1:10.43	Strong flexing of femur towards thorax	
Pattern 1			
Pattern 2			
Pattern 3			
Pattern 4			
Pattern 5			

Exercise 3B: Stimulation of the metathoracic ganglion

Now that you have the spiking patterns of motor input from the metathoracic ganglion to the legs, you will test whether the patterns you recorded will lead to the specific movements you observed.

1. If using the alligator clip microstimulation electrode, attach one electrode to the recording electrode already placed in the metathoracic ganglion. If using electrode pin attached to an audio cable, replace the recording electrode with the microstimulation electrode. Try to insert the electrode into the same spot.

2. Similar to Exercise 1, begin measuring the effectiveness of your microstimulation electrodes. In Table 3, record the effect of microstimulation on your cockroach prep in terms of movements. Record a brief description of movement in response to a 5 second stimulation.

Table 3. Effect of Frequency and Amplitude on Movement

Frequency	Min Output	$\frac{1}{2}$ Output	Max Output
20 Hz			
80 Hz			
200 Hz			
1000 Hz			
2000 Hz			

3. Now, using the Spike Patterns generated in Exercise 3A, stimulate the metathoracic ganglia at different volumes. Record the results in Table 4. Describe the movement observed (if any), and whether this movement is the same as the corresponding movement recorded in Table 2.

Table 4. Effect of Spike Patterns and Amplitude on Movement

Spike Pattern	Min Output	½ Output	Max Output
Pattern 1			
Pattern 2			
Pattern 3			
Pattern 4			
Pattern 5			

DISCUSSION QUESTIONS

1. In this experiment you tested the responsiveness of the cockroach legs to a wide range of frequencies and amplitudes. Was there any commonality in the types of frequencies and/or amplitudes that caused the most responsiveness? Why do you think that might be?

2. Were you able to identify any spiking patterns that led to motor movements? If so, what were the characteristics of these patterns? What types of movements were you able to find these patterns for?

3. When you stimulated the metathoracic ganglia with spike patterns that you had observed during leg movements, did it generate any leg movements? Were these leg movements the same as the ones you had observed while recording those spike patterns? Why do you think this is?

LAB SIX: SPIKE REFERENCING

OVERVIEW

In this lab you will:

1. Learn about voltage, and the practical application of measuring a voltage differential;
2. Consider how “incorrect” electrode placement can lead to “incorrect” experimental results;
3. Investigate the importance of ground electrode location to the quality of neural recordings.

OBJECTIVES

Before doing this lab you should:

- review action potential mechanisms and the structure of neurons;
- study the anatomy of the nervous system within the cockroach leg.

After doing this lab you should be able to:

- explain the concept of voltage, and what we really mean when we give a voltage value;
- describe what types of recording results would be expected based upon ground electrode placement in different locations;
- describe the method you would implement to find the best ground location for a given recording location.

EQUIPMENT

- SpikerBox
- Computer with Audacity installed or iPhone/iPad/Android with Backyard Brains app
- Associated laptop cable or iPhone/iPad/Android cable
- Cockroach
- Dissection scissors
- Toothpick or skewer that is broken in half so one end is pointy

INTRODUCTION

In order to understand this experiment, one must first have an understanding of **volts** (or Voltage). Voltage is defined as the electric potential energy per unit charge, and is measured in joules per coulomb (1 volt = 1 joule/coulomb). Specifically, Voltage is a measure of the electrical **difference** between two points

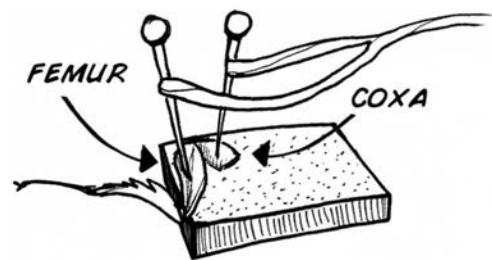
For example, you have ever combed your hair on a dry day and noticed your hair begin to “float” towards your comb? This is because you have caused an electrical difference between your comb and your hair.

When you comb your hair, the plastic comb “strips” electrons from your hair, making a difference in charge, or “voltage,” between your hair and the comb. As a result, the separation in charge curiously makes your hair attracted to the comb, while also making individual hair strands repel each other.

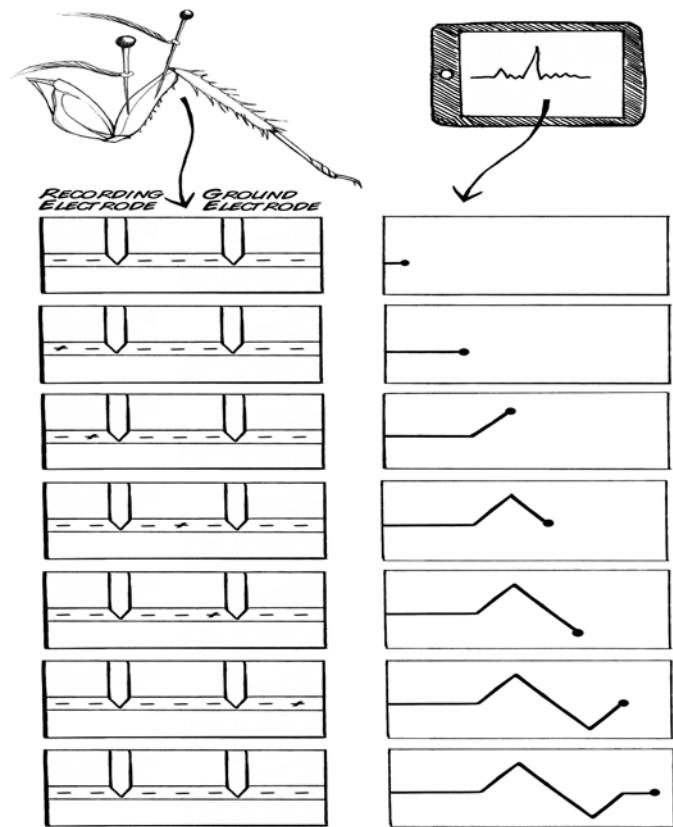
This difference in charge also means you can never just measure the voltage at a single point. Any voltage value that you see will always be a relative value, or, a value at one point (comb) as defined with respect to the value at another point (hair). Your hair and comb have a relative difference in charge, with the comb slightly less negative than the hair. As another example, the nine volt battery which powers your SpikerBox has a 9V difference in voltage between the ‘+’ and the ‘-’ ends.

Because voltage is a measure of the difference between two points, your SpikeBox electrode cable has two needles instead of one; we are measuring the *voltage* between two electrode pins. In an ideal world, your two electrodes have distinct identities, a “recording” electrode that captures the signal of interest (spikes) and is hopefully near nerves, and a “ground” electrode that ideally is in a part of the organism that has little electrical signal present.

Why? Because you need a difference between the two electrodes to see your spikes. Spikes (action potentials) are exceedingly small, on the order of microvolts to millivolts, and must thus be amplified by devices like your Spikerbox in order to be detected.



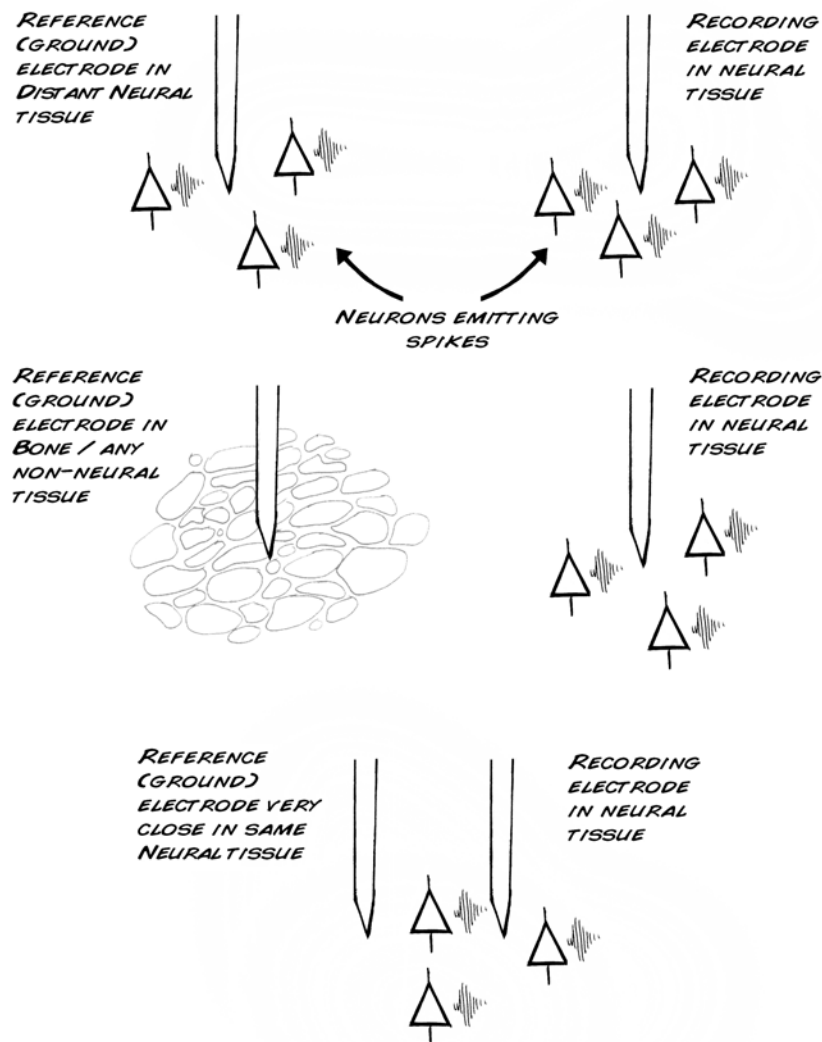
Let's consider what happens when a spike travels down a nerve, and we have our recording and ground electrodes on opposite ends of the nerve. In the figure to the right, the spike is represented as a '+' on a surface of all '-'s within the nerve. As the recording electrode encounters the spike, the result is an upward slash on our voltage-measuring scope. Then, when the spike is in between both the recording electrode and the ground electrode, there is no difference between what each electrode "sees", and we measure a zero. As the spike then travels past the ground electrode, the recording electrode now seems negative with respect to ground, and there is a downward slash of the scope. Then, when the spike travels further down the nerve away from the ground electrode, the voltage reading returns to normal.



Now consider a thought experiment where we encounter a strange, alternative world where action potentials travel infinitely fast down a nerve. Imagine we try to record spikes from this nerve with the same electrode configuration as above. If an action potential happens, and we are measuring the voltage difference between the recording electrode and ground electrode, what do we expect to see?

Since both the recording electrode and the ground will be at the same electrical potential due to the infinitely fast action potential, we would measure a zero value...which would leave us to hypothesize that this animal does not have action potentials, and thus uses some new, unknown to science, method of neural communication! This would be great for our careers if we were correct, but unfortunately our conclusions would not be true. Our electrode placement simply did not allow us to "see" the action potential.

Thus, since a voltage measurement is a measurement of the difference between your recording and ground electrodes, you need to think carefully about where you place your electrodes when recording neural signals. For example, consider the following three conditions:



In the top condition (electrodes far away, but both in neural tissue), you are recording a “very crowded” mix of spikes from six neurons (as you would see the three neurons near the ground electrode and the three neurons near the recording electrode). In the middle condition (ground electrode in bone, recording electrode in neural tissue), your recording would be less “crowded”, as the ground electrode is in bone where no neurons are present. You would only see spikes from the three neurons near the recording electrode. Now consider the bottom condition. Notice there are two neurons exactly in between the recording and the ground electrode. Both electrodes would see the exact same signal from these two neurons; therefore you would not be able to observe the middle neurons. You would, however, be able to record from the neuron on the right side that is closer to the recording electrode. In this recording, you would get a very clean view from only 1 neuron, something we neuroscientists love. And why? We shall return to that later.

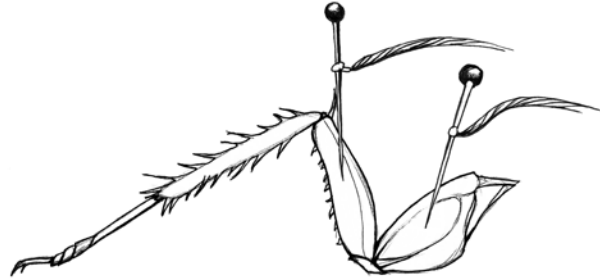
But for now, in this experiment, we will examine various configurations of recording electrodes and ground electrodes.

PROCEDURE

Note: for this experiment we refer to “ground” and “recording” electrodes but you can arbitrarily decide which of your electrodes you will call a recording electrode and which one you will call a ground.

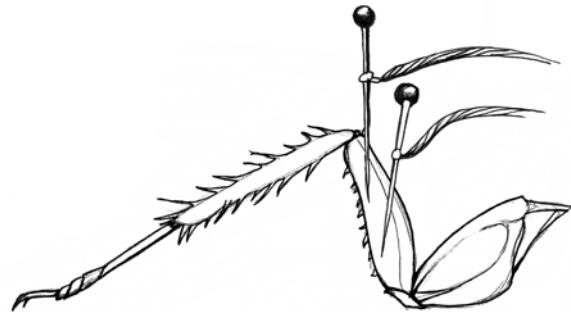
Test the quality of your recordings in the femur with your ground in the coxa.

1. Set up your computer/mobile device for recording and prepare a cockroach leg as described in Experiment 1.
2. Put your recording electrode in the femur, and your ground (reference) electrode in the coxa as shown in figure to right.
3. Blow on the leg of your cockroach. Make note of whether the response is a broad “whoosh” of neural activity, or whether it is an individual spike train.
4. Carefully touch the barbs on the leg with a toothpick. Make note of whether the response is a “whoosh” or an individual spike train.



Test the quality of your recordings in the femur with your ground in the femur

1. Repeat the same procedure as above, but Move your ground electrode from the coxa into the femur, as shown in figure to right.



Test the quality of your recordings in the coxa with your ground in the coxa

1. Move both electrodes into the coxa, as shown in the right, and repeat the same observations as above.



DISCUSSION QUESTIONS

1. What is voltage? When voltage is reported, is it an absolute number or is it a differential value?

2. In this experiment, you learned about what happens when you move your ground and recording electrodes to a new location. What do you think would happen if you reversed your 'ground' and 'recording' electrodes in the first configuration, moving the 'ground' to the femur and the recording electrode to the coxa? Would you observe any difference?

3. With one electrode in both the coxa and the femur, there is generally a lot of “background activity”. How does that change when you put both electrodes into the femur? Can you provide an explanation for the difference? Here is a [classic cockroach leg neuroanatomy paper](#) from 1954 that can help.

4. On page three of this exercise, we talk about recordings with respect to the different ground conditions (in distant neural tissue, in bone, in close neural tissue, etc). Draw below what the recordings would look like in these conditions, using the format of the figure on page 4. Note, one of the conditions in your experiment is not represented in the illustration.

5. Describe the method that you would implement to find the best ground location for a given recording location?



LAB SEVEN: NEUROMODULATION

OVERVIEW

In this lab you will:

1. learn how to extract neuromodulating agents from common products;
2. test how these agents affect neuronal spiking patterns; and
3. investigate how different classes of neuromodulators affect various populations of neurons in the cricket.

OBJECTIVES

Before doing this lab you should understand:

- the basics of cricket anatomy
- how neuromodulators affect a synapse
- how the differential expression of receptors can alter neuronal responsiveness

After doing this lab you should be able to:

- explain neuropharmacology and neuromodulation
- describe how alcohol, glutamate, and nicotine affect neuronal firing rates
- design an experiment to test how a substance may affect the nervous system

EQUIPMENT

- SpikerBox with audio cable adapter
- computer with Audacity installed
- crickets
- ice water or access to a freezer
- dissection scissors
- toothpick
- 3" petri dish
- corrugated cardboard (cut to fit into petri dish)
- plastic pipettes
- distilled H₂O, NaCl, baking soda, MSG, ethanol, tobacco
- 1 cc (1 ml) syringes

INTRODUCTION

About Anuradha Rao

This experiment is dedicated to Anuradha Rao, a neuroscientist who studied pharmacology and enjoyed educational outreach. Her memorial fund generously allowed Backyard Brains to present experiments and prototypes at the 2010 Society for Neuroscience Conference in San Diego, CA.



In this experiment we will test the effect of neuroactive compounds on central nervous system neurons. Obtaining drugs that affect neurons can be quite difficult, as they are often very dangerous (like the batrachotoxins of poison dart frogs or the tetrodotoxins of fugu puffer fish, both of which block sodium channels) or are drugs of abuse (like cocaine, which allows dopamine to stay in synapses longer than normal).

Also, the blood-brain barrier (BBB) prevents the passive diffusion of many substances into the brain. The BBB is composed of brain endothelial cells that are packed closely together around all capillaries, with tight junctions and a higher than normal electrical resistance ($2000 \Omega/\text{cm}^2$ as opposed to the usual $5 - 10 \Omega/\text{cm}^2$). This barrier ensures brain function in vertebrates and insects by maintaining the ionic integrity inside the brain. It

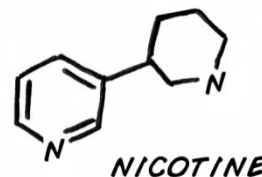


BBB image courtesy of Wikipedia

prevents many toxic substances from entering the brain, protects brain neurons from circulating neurotransmitters such as norepinephrine, and restricts the diffusion of objects such as bacteria or hydrophilic molecules into the cerebrospinal fluid (CSF). Even K^+ ions are excluded. The barrier does allow the diffusion of small hydrophobic molecules (e.g. O_2 , some hormones), and specialized barrier cells actively transport some metabolic products such as glucose across the BBB with specific proteins. The practical consequence of the BBB on our experiments is that only certain drugs with specific characteristics can be used to influence neurons. Those also makes designing drugs to treat brain function very difficult for pharmaceutical companies.

However, we do have access to some neuroactive compounds that we can use on our insects: nicotine, monosodium glutamate, and ethanol.

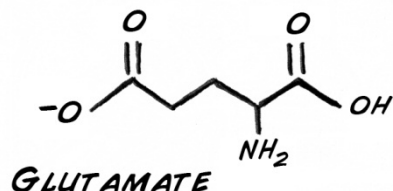
Nicotine comes from the tobacco plant. Tobacco evolved nicotine to prevent insects from eating its leaves. Nicotine is a powerful acetylcholine receptor **agonist**; it amplifies the effect of acetylcholine (ACh) binding to its receptors in synapses, causing a neuron to fire more (due to increased



sodium ion influx).

Whereas nicotine is a drug that acts on receptors that neurotransmitters bind to, monosodium glutamate itself is a neurotransmitter. Once dissolved in water, it turns into positively charged sodium ions and negatively charged glutamate ions. Glutamate is normally part of the metabolic pathway of glycolysis (breakdown of sugar) and is readily available from the foods you eat.

In fact, over 80% of the synapses in your brain use glutamate as it is an excitatory neurotransmitter. Do you think glutamate will be excitatory in an insect as well?



Ethanol is a drug that also is an agonist to neurotransmission, however it acts to increase the effect of GABA binding channels. Unlike ACh receptors, stimulation of GABA channels often leads to inhibition of action potentials. In other words, GABA channel stimulation will slow down the firing of a neuron. Stimulation of human neurons with ethanol can slow them down; will the same happen in an insect?

PROCEDURE

Exercise 1: Preparation of Neuroactive Solutions

Saline Solution: Create a simple saline solution by combining 1.5 g table salt (NaCl) and 1.25 g baking soda in 250 mL of distilled water.

Nicotine: To create your nicotine solution, take a cigarette or small cigar, remove all the shredded tobacco leaves, and place them in a small container (a clear pill bottle, for example). Fill the container with saline solution, put the cap on, shake up the mixture, and allow it to sit for a couple days to extract the nicotine. Over time the solution should turn yellowish-brown. If you are in high school, your teacher may have already prepared this solution.

Glutamate: To create your glutamate solution, you will use the food additive monosodium glutamate (MSG). You can find this at an Asian Import grocery store, as MSG is a common flavor enhancer in Asian foods. Fill up a clear pill bottle about a quarter full of the MSG salt crystals, fill the remainder of the bottle with saline solution, and shake thoroughly to dissolve the MSG. Note that not all of the MSG will dissolve, as you are making a saturated solution.

Ethyl Alcohol: Mix 196 mL of saline with 4 mL ethanol to make a **2% EtOh solution**. Take 12.5 mL of your 2% EtOh solution, add 87.5 mL of saline to make a **0.25% EtOh solution**.

Exercise 2: Cricket Cercal Ganglion Preparation

For this exercise, you will be using measurements from the cricket cercal system to determine the effect of a drug on spiking patterns and rates.

Note: This experiment requires crickets to be sacrificed

1. Place your crickets in a freezer for 5 minutes. When the cricket has stopped moving and has been anaesthetized, place it on your lab bench. Take care to limit the amount of time your crickets are in the freezer, as they are more sensitive to cold than the cockroaches used previously. If 5 minutes is not enough, return the crickets back to the freezer and check on them in one-minute intervals.
2. Using your dissection scissors, decapitate the cricket at the junction between the head and the thorax. Place some petroleum jelly on the cut.
3. Place the cricket into a petri dish with a small piece of cardboard placed on the bottom. The cardboard will allow you to hold the cricket in place with the SpikerBox electrodes while applying liquids to the preparation in a clean fashion.
4. Insert your SpikerBox recording electrode through the **abdomen** of the cricket into the **cercal ganglion**. The cercal ganglion is the most posterior ganglion in the ventral nerve cord (VNC). Try to place the electrode into the center of the ganglion. Stick the ground electrode into the thorax where the head used to be. Now, using a small syringe (you can buy these at your local pharmacy), inject a few drops of **saline solution** into the abdomen of the cricket.
5. Using a toothpick or by blowing, stimulate the cerci of the cricket. This should generate a strong spiking pattern and possibly a stereotypical writhing movement of the abdomen.
6. Turn on your SpikerBox and Audacity. Connect your SpikerBox to your computer and begin recording. Plug your SpikerBox into your computer, turn it on, and start Audacity. **Adjust the Y-axis** of the recording so that the spikes observed are maximized. **Adjust the X-axis** (using the magnifying glass tool in the menu bar) so that you can differentiate bursts directly related to stimulation of the cercal ganglion.
7. If you do not observe movement or spiking in response to stimulation, be patient. It may take several minutes for the cricket's VNC to warm to an optimal recording temperature. In this time period, activity measured from the cercal ganglion may seem quite low. If, after 5 minutes of warming, you are still unable to observe any spikes, adjust your electrodes and repeat stimulation. If you are unable to observe spikes after another few minutes of stimulation, you may need to use a new cricket. Once you have established

consistent recordings, you are ready to begin observing the normal firing pattern of the cricket cercal ganglion.

Exercise 3: Control Cercal Response

1. You will now proceed to stimulate the cerci of the cricket by blowing from either the right or left side of the cricket. Begin recording on Audacity.
2. As one function of the cercal system is to differentiate directionality of a wind stimulus, you should be able to see differences in response to your blowing from one side versus the other. Using Table 1, record the time and duration of stimulus (blowing) and the side the stimulus was delivered (right or left). Repeat this twice for each side of the cricket.
3. Stop recording on Audacity.
4. Using the times recorded in Table 1, highlight the cercal responses to your stimulation. Amplify each of the highlighted trace using **Effects-Amplify** in the menu bar. Do not amplify the entire recording, just the cercal responses.
5. In Table 1, diagram the spiking pattern of each trace and give a brief description of any movements generated in response to your stimulation.

Table 1. Control Cercal Responses

Trace	Time (Duration)	Left/Right	Pattern and Brief Description of Movement
1			
2			
3			
4			

Exercise 4: Testing Neuroactive Compounds

1. Beginning with the nicotine solution, you will now test the affect of pharmacological agents on the cricket cercal response. Using a plastic pipette, remove any excess saline from the recording electrode. Begin recording with Audacity.
2. Now, using your small syringe, inject a few drops of **nicotine solution** into the abdomen of the cricket.
3. After a minute (to let your solution reach the cercal ganglion), stimulate the cerci as you did in Exercise 3. This may take several attempts. Record in Table 2 the time for two successful right and left cercal stimulations. Stop recording.
4. Using the same procedure as Exercise 3, amplify and sketch the traces of your successful stimulations. Include a brief description of any movements that appear to have been generated in response to your stimulation.

Table 2: Effects of Nicotine on the Cricket Cercal Response.

Trace	Time (Duration)	Pattern and Brief Description of Movement
Left Side 1		
Left Side 2		
Right Side 1		
Right Side 2		

5. After you have finished with your nicotine solution, wash out the recording electrode with saline.
6. Repeat steps 1-5 with the Glutamate and Ethanol solutions, taking care to wash out residual solutions with saline in between trials. Record your results in Tables 3-5 below.

Table 3: Effects of Glutamate on the Cricket Cercal Response.

Trace	Time (Duration)	Pattern and Brief Description of Movement
Left Side 1		
Left Side 2		
Right Side 1		
Right Side 2		

Table 4: Effects of 0.25% Ethanol on the Cricket Cercal Response.

Trace	Time (Duration)	Pattern and Brief Description of Movement
Left Side 1		
Left Side 2		
Right Side 1		
Right Side 2		

Table 5: Effects of 2% Ethanol on the Cricket Cercal Response.

Trace	Time (Duration)	Pattern and Brief Description of Movement
Left Side 1		
Left Side 2		
Right Side 1		
Right Side 2		

DISCUSSION QUESTIONS

1. Based upon your results, which pharmacological agents led to an increase in cercal activity? Was this effect consistent on both sides of the cricket? Explain.

2. Based upon your results, which pharmacological agents led to a decrease in cercal activity? Was this effect consistent on both sides of the cricket? Explain.

3. Did any pharmacological agent affect the duration of the cercal response? If so, which? Why might this be the case?

4. Describe one significant difference in the spiking pattern observed between your control recordings and an experimental condition. What might these differences reflect?

5. Were there any differences in the movements observed between your control and experimental conditions? What might explain this?

LAB NINE: NeuroProsthetics

OVERVIEW

In this lab you will:

1. Learn about the characteristics of the nervous system that make neuroprosthetics a viable form of treatment for pathologies that interrupt the flow of neural information;
2. Observe the differences in scale between the magnitude of a recorded neural signal and the magnitude of stimulation that would be required to mimic that neural signal;
3. Test what effect a stimulation pattern that mimics a recorded biological signal from one limb can have on a second limb.

OBJECTIVES

Before doing this lab you should:

- review what happens in neural pathways during damage;
- study how electrodes interface with the nervous system during both recording and stimulating, and how the biological signal can be transduced (changed) to a form that a computer can use;
- learn about the current state of the art for brain-machine interfaces.

After doing this lab you should be able to:

- explain the difference between “biological” signal transmission and “machine” signal transmission;
- describe any differences in scale between the magnitude of the neural signals recorded and the required magnitude for a stimulation response, and why we might see those differences;
- design an experiment to test whether the recorded neural signal from moving one cockroach leg can be used as a stimulating signal to make a second leg move in the exact same way.

EQUIPMENT

- One SpikerBox
- Cockroach (two cockroach legs)
- Dissection scissors
- Toothpick or skewer that is broken in half so one end is pointy
- Two RadioShack Amplifier/speakers
- One Y-splitter
- Two “Patch” or laptop cables
- One stimulation cable

INTRODUCTION

In experiment four we learned that our somatosensory system is a complex, multi-level network that includes receptors that interface with the outside world and generate an initial signal, nerves that carry that signal to the spinal cord, the cord that carries the information to the brain, and the brain that processes the info and decides what to do with it. This type of multi-level relationship is actually the basis of all of our senses, from vision (eyes to optic nerve to brain) to hearing (ears to auditory nerve to brain). The same is true for the opposite of sensing...Motor Control! Motor control also requires multiple levels of the nervous system working together to initiate movements. In the case of motor activity, though, the signal begins in the brain then travels down the spinal cord until it eventually reaches the muscles the brain wants to move. Thus, both healthy sensing and healthy motor function require an uninterrupted nervous connection between the brain and the peripheral input/output organs. And when that neural pathway is damaged, the resulting deficit is characterized by the loss of function...lack of vision is blindness, lack of hearing is deafness, and lack of ability to sense or control the legs is termed paraplesia (or paralysis), etc.

When a mammal breaks a bone, if the bone is set correctly it will often re-knit into a healthy bone. When a mammal tears a muscle, if given time to rest and recover the muscle will generally re-knit into an even stronger muscle (this is the foundation for weight training). However, in a continuing mystery to science, neurons in the mammalian brain and spinal cord do not regrow when damaged, save for the exception of a small population of neurons in the memory (hippocampus) and smell (olfactory) parts of the brain. Thus, given current technology, when damage occurs to the brain or spinal cord, sometimes engineers and doctors try to bypass the site of injury through electronic and computer means with devices called **neuroprosthetics**.

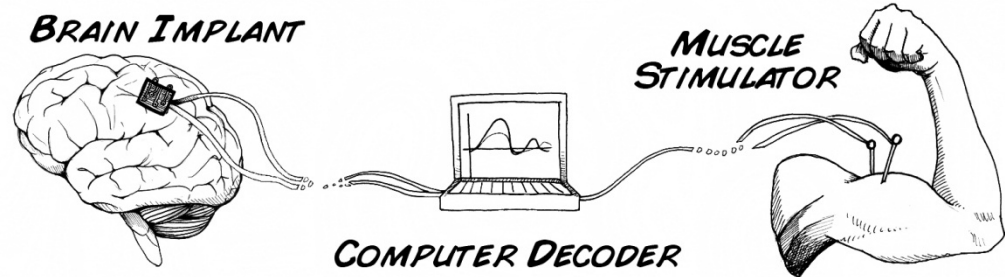


Mature “Hipster” neurons won’t regrow/divide when damaged.

Perhaps the easiest way to visualize a neuroprosthetic is to picture some famous storylines in science fiction movies or comic books. In “Empire Strikes Back”, Luke Skywalker had a robotic hand installed after Darth Vader cut it off, and Luke could control his hand simply by thinking about it. In “Star Trek, The Next Generation”, Geordi LaForge was a blind man that could see through a machine visor that interfaced with his brain. In the X-men movies, Professor Xavier had a huge machine, Cerebro, that interfaced with his brain through a helmet and enhanced his natural mental abilities.

So, what do these examples all have in common? In each of them, a device interfaced with the nervous system to replace or enhance the function of a body part. This, then, is a working definition for a neuroprosthetic. And neuroprosthetics are no longer limited to science fiction...they have made their way into the real world. For example, if someone breaks their spinal cord above a certain point, they are confined to a wheelchair. But the muscles in their legs are still alive, and the brain can still initiate the command to move their legs.

The message, however, cannot travel from the brain to the leg muscles because the spinal cord is damaged.



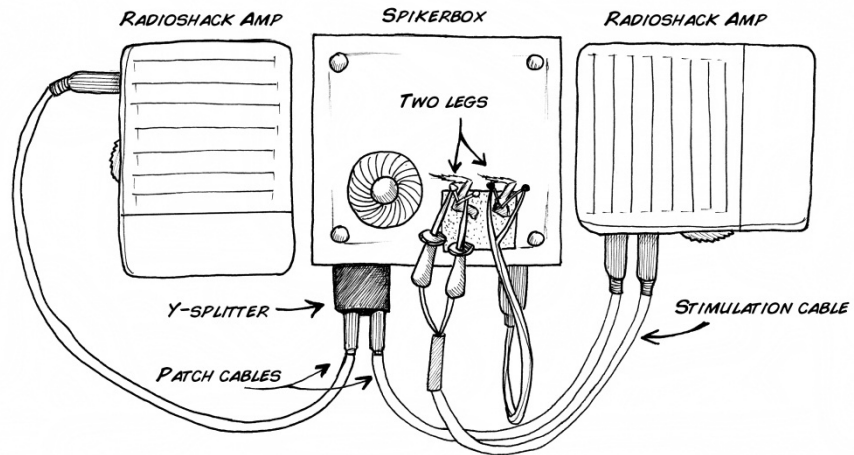
If there were a way to “read out” the command signal from the brain and use it to stimulate muscles or a robotic limb, such a device would be a neuroprosthetic. As we’ve seen in our experiments thus far, we can use electrodes to interact with the signals that are found in neurons. Action potentials are initiated and propagated through changes in cellular ionic concentrations, and the signal travels as charged ionic current. When an electrode is near a neuron, the charged ion currents induce electrons to move on the surface of the electrode that generates a similar magnitude current on the metal. And when the electrode is attached to a machine like your Spikerbox, the machine can then “read out” the neural signal. A [research group in Pittsburgh](#) has used this exact neural recording technique to identify the command signals in a monkey brain that initiate movement, and then trained a monkey implanted with electrodes in his brain to control a robotic arm to feed itself. We will study a very basic neuroprosthetic preparation in this experiment.



PROCEDURE

Exercise 1: Use the recorded neural signal from one cockroach leg to modulate the other

1. Prepare cockroach legs as described in Experiment 1. Cut off two of the cockroach legs for use in this experiment.
2. Place the two cockroach legs on the cork of your SpikerBox, and hook up one leg to the SpikerBox in the standard way as in Experiment 1.
3. Plug the Y-splitter into the SpikerBox.
4. Plug the two patch cables into the Y-splitter.
5. Plug the two Radio Shack Amplifier/Speakers into the other ends of the two patch cables. One amplifier/speaker is for simultaneously listening to the spikes while doing your experiment, and the other amplifier/speaker is for stimulating the muscles of the second leg.
6. Plug the Stimulation Cable into one of the amplifiers
7. Place two needles into the second cockroach leg, and hook up the stimulation cable to these two needles
8. Turn on one of the amplifier/speakers to hear the spikes, and then turn on the second amplifier (the “stimulating” amplifier).
9. After the brief initial movement in the stimulated leg (due to capacitive discharge when the amplifier is turned on), slowly turn up the volume on the stimulating amplifier until the leg starts twitching. Then, turn the volume down below this point (bringing the leg just “below threshold”).
10. Begin brushing the leg that is hooked up to the SpikerBox.
11. Make note of any changes in the leg that you brush (the “recorded” leg)
12. Make note of any effects that you note on the “stimulated” leg when you brush the “recorded leg”



DISCUSSION QUESTIONS

1. In this experiment, we plugged two secondary amplifiers into the SpikerBox. One amplifier was simply to allow us to hear the spikes and is not that important. However, the second amplifier was needed to amplify to spikes to a high enough (approximately 2 volts) voltage to stimulate the second leg. What does this say about the difference between the size of the neural signals that we are recording, and the size of the stimulation signal required to effect a change in the leg? What is the explanation for this difference?

2. From previous experiments, we learned that neural signals travel through the nervous system using action potentials (spikes). What are the charge carriers that generate action potentials in the body called (hint: Na^+ , K^+ , Cl^- are examples of these)? When current travels through electrodes and computers, the charge carrier is different. What is the name of the charge carriers for electricity? Since the charge carriers are different, how do electrodes interface with the body?

3. In this experiment, what effect did brushing the “recorded” leg have on that leg? What effect did brushing the “recorded” leg have upon the “stimulated” leg? How would you design an experiment to test whether the recorded neural signal from manually moving one cockroach leg could be used to make a second leg move in the exact same way? Based on the results of the present experiment, do you believe that the proposed/hypothetical experiment would work perfectly in a human? Why?

Lab Nine: Oxygen and Spiking

OVERVIEW

In this lab you will:

1. Learn about the importance of oxygen in generating neural action potentials;
2. Observe what happens to neural spikes when the system is deprived of oxygen; and
3. Test how long neurons can be “starved” of oxygen but still recover, and at what point the oxygen starvation is irreversible.

OBJECTIVES

Before doing this lab you should:

- review what you learned in Experiment 6 about neuron energy requirements for generating action potentials;
- study what it means for an organism to be “aerobic”, and why oxygen is necessary for their survival;
- learn some of the basics of how biochemical equations describe events that are important for life.

After doing this lab you should be able to:

- explain why neurons stop generating spikes in the absence of oxygen, and what happens when oxygen is returned again;
- describe whether the “time it takes for the spikes to go away” in the absence of oxygen and the “time it takes for the spikes to come back” once oxygen returns is the same or different;
- design an experiment to test whether a different coating (besides petroleum jelly) can prevent the cockroach leg from drying out while still allowing oxygen to pass through.

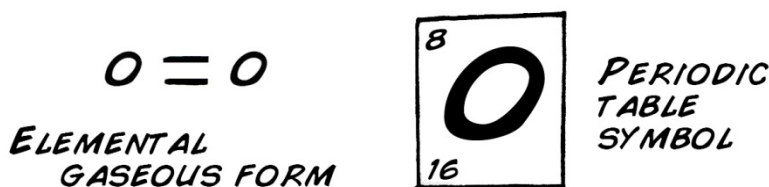
EQUIPMENT

- One SpikerBox (though you will find this experiment easier with two)
- Computer with Audacity installed or iPhone/iPad/Android with Backyard Brains app
- Associated laptop cable or iPhone/Android cable
- Cockroach (two cockroach legs)
- Two recording electrodes
- Dissection scissors
- Toothpick or skewer that is broken in half so one end is pointy
- Petroleum Jelly
- Stopwatch

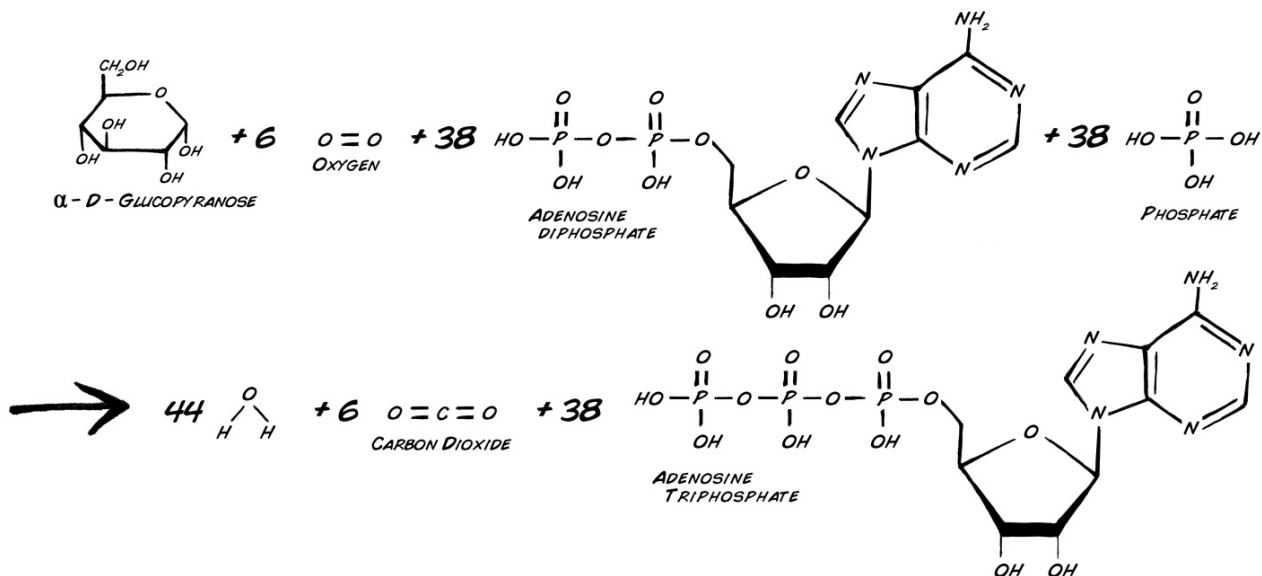
INTRODUCTION

In Experiment 6 we talked about the energy that neurons need to continue generating spikes and specifically about the energy needed to operate the sodium-potassium pump. This energy is provided by adenosine triphosphate (ATP), which is made largely from the foods that you eat. Food, though, is only one of the things that your body requires to stay alive. What is invisible, tasteless, void of form, but yet critical to your life?

Oxygen! Oxygen is an “oxidizer”, meaning it is greedy for electrons. Pure gaseous elemental oxygen is so electron greedy that it was a poison for most life on Earth billions of years ago. In fact, the only elements greedier for electrons are fluorine and chlorine, both of which are still very poisonous in elemental gas form. Once life forms evolved to harness the oxidizing nature of oxygen, though, aerobic (oxygen breathing) creatures became the dominant organisms on the planet.



All animals, including insects, fish, birds, dogs, and you, need oxygen in addition to food to generate enough ATP to stay alive. The reasons and mechanisms for why oxygen is so vital to energy generation can be explained using biochemistry.

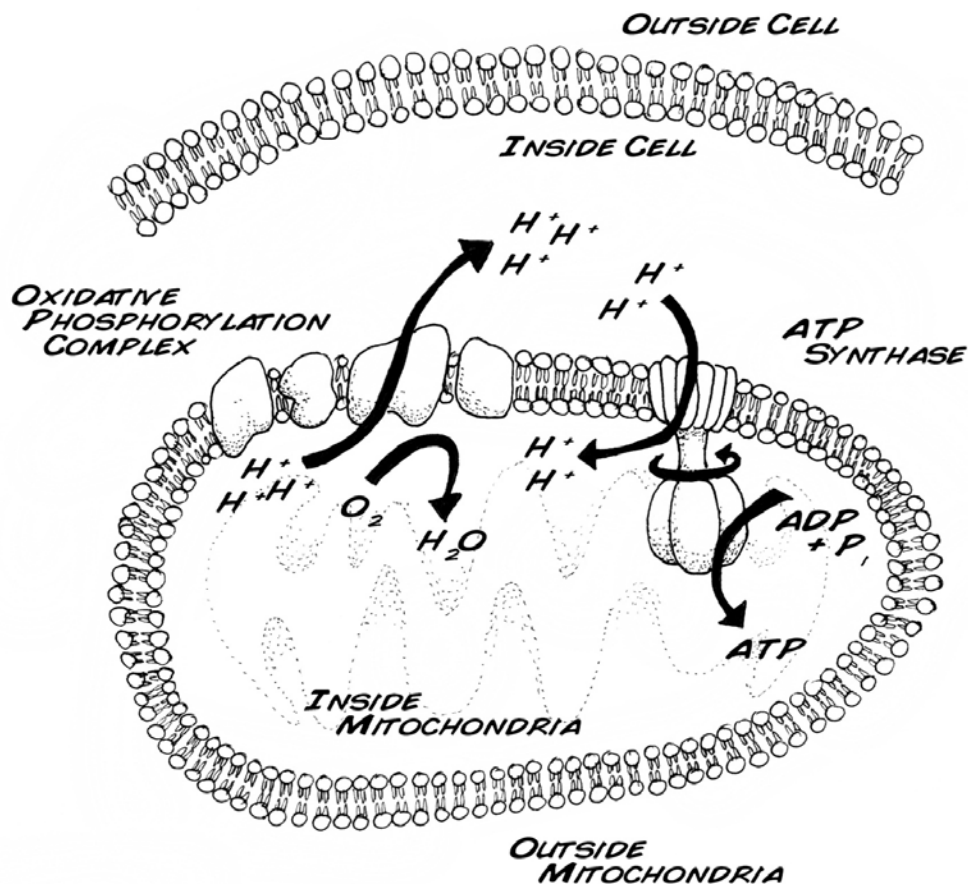


This complicated chemical reaction has dozens of tightly controlled steps that are classified into three different pathways:

1. Glycolysis
2. The Krebs cycle
3. The oxidative phosphorylation pathway

Glycolysis is the breakdown of the 6 carbon sugar into 3 carbon pyruvate. The Krebs (citric acid) cycle generates electron-rich molecules with funny names like NADH and FADH. The oxidative phosphorylation pathway is where the electrons from NADH and FADH are transferred through multiple molecules (with more funny names like coenzyme Q and complex IV) before going to the final electron acceptor, oxygen.

Oxygen is ultimately converted to H_2O (water). As these electrons (which began with NADH and FADH) move through successive owners (like coenzyme Q, complex IV, and oxygen) in the oxidative phosphorylation pathway, this creates energy that is used to generate an excess of hydrogen ions (protons) outside the membrane of the mitochondria of the cell. The mitochondrion is the cell organelle where this oxidative phosphorylation pathway occurs. The excess protons outside of the membrane produce an ionic imbalance, which is used to power a very special enzyme: ATP synthase. The excess hydrogen ions then travel back into the mitochondria by going through the ATP synthase, which causes the synthase to “rotate”, which generates the work that converts ADP into ATP (through protein chemistry). This process is illustrated in the figure below.



Take home message:

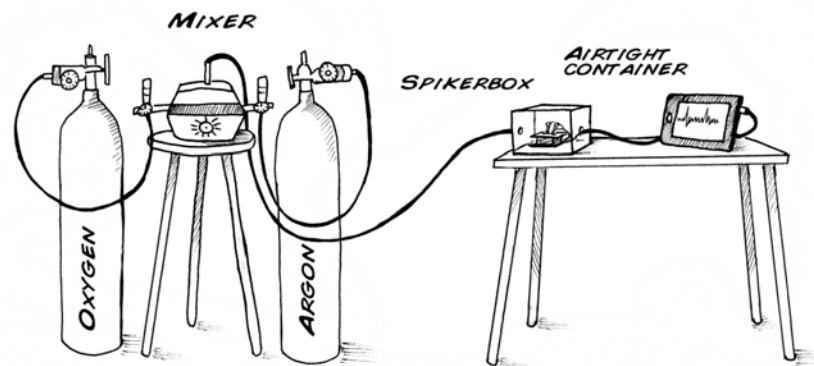
- a) These three reaction pathways are necessary to break down the sugar into a form that the body can use
- b) Two of the processes require oxygen in order to work (glycolysis doesn't require oxygen)
- c) One of the main outputs of these chemical interactions is ATP
- d) ATP, as we learned in experiment 6, is the key energy generator required for neurons to maintain ionic balance across their membranes

Thus, without oxygen there is not enough energy for the body.

Now, let's think back to how this applies to the cockroach legs that we record from. While the disconnected leg has enough food stored inside to survive for up to a couple of days (as we saw in Experiment 6), the leg does not have enough oxygen to survive for nearly as long. This same holds true for you...think how long you can go without eating, then compare that with how long you could survive without breathing. Big difference, huh? So, let's see if we can investigate more specifically what the relationship is between oxygen and spike generation in the cockroach leg. And since we've been doing experiments for a while now, let's take this opportunity to compare different ways to experimentally test the same concept and use our judgment on which approach makes the most sense for us to use.

One way to test this experimentally is to put your cockroach leg and SpikerBox in a positive airflow container. To do this, you would need to buy an oxygen tank and an argon tank (argon is typically used as an inert gas and would be the gas that we replace oxygen with in this experiment). You would also need some flow regulators and tubing, and you would then have to hook up the tanks such that you can slowly decrease the percentage of oxygen in the airtight container to see the effect that different levels of oxygen has on spiking activity in the cockroach leg. This experiment would give us a very detailed relationship between oxygen and spike generation but would be expensive and difficult to do, especially outside of a formal lab setting. So, let's call this proposed experiment "the hard way".

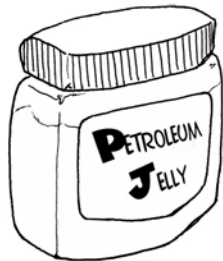
HARD WAY



Thankfully, there is an alternative approach we can take. Since the leg of the cockroach gets all of its oxygen through the open wound (we discovered this when [we visited our friendly local electron microscopist](#)), we can “starve” the leg of oxygen by simply liberally coating the leg with petroleum jelly (vaseline). We’ll call this approach “the easy way” to do it, and continue with that approach for the procedure of this experiment.

EASY WAY

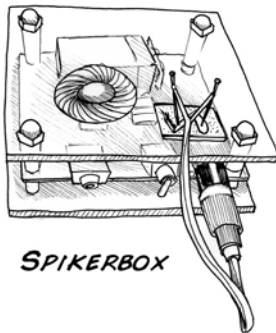
PETROLEUM JELLY



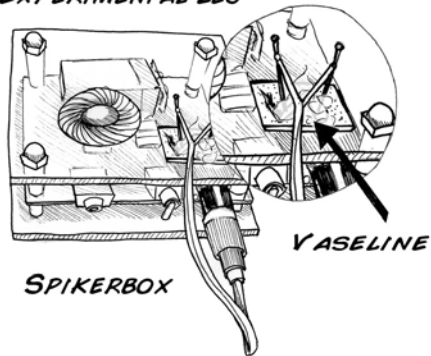
STOPWATCH



CONTROL LEG



EXPERIMENTAL LEG



PROCEDURE

1. Set up your computer and prepare cockroach legs as described in Experiment 1. Cut off two legs for this experiment. (Note: you can use two SpikerBoxes (one for each leg), but you don't have to. It is possible to do this experiment with only one SpikerBox; you could simply swap out the electrodes that are hooked up to each cockroach leg)
2. Liberally coat one leg (the "experimental leg") with petroleum jelly using a toothpick, and start your stopwatch. The "control leg" is the other leg and the one with no petroleum jelly on it.
3. Every 10 – 15 minutes, manipulate both the control and the experimental leg by touching the barbs with a toothpick to see if you can evoke spikes.
4. After a time, the experimental leg should stop spiking. Mark down the time that this occurs on the chart. (Note: if the experimental leg doesn't stop spiking, you may not have put enough petroleum jelly on the leg to create a good seal against the air. If this happens, put on more jelly and re-start your stopwatch).

	<i>SPIKE FAILURE TIME</i>		<i>SPIKE RESTORE TIME</i>
<i>LEG 1</i>	_____	<i>ROUND 1</i>	_____
	_____	<i>ROUND 2</i>	_____
<i>LEG 2</i>	_____	<i>ROUND 1</i>	_____
	_____	<i>ROUND 2</i>	_____
<i>LEG 3</i>	_____	<i>ROUND 1</i>	_____
	_____	<i>ROUND 2</i>	_____

5. Carefully remove the petroleum jelly with a napkin or paper towel. Restart your stopwatch. Every 10 – 15 minutes, touch the barbs of both legs to attempt to evoke spikes. Eventually, the spikes should return to the experimental leg. Mark the time at which the spikes return on the chart above.
6. Repeat steps 2 – 5 as long as you have time for AND as long as the control leg is still generating spikes.
7. Repeat the experiment over a couple of legs to see if you can find an "average time for the spikes to go away" and an "average time for the spikes to come back".

DISCUSSION QUESTIONS

1. It's easy to understand why the spikes eventually go away when you starve the leg of oxygen, but why do the spikes come back? How long can the leg be coated in petroleum jelly before he spikes go away permanently?

2. When doing this experiment, you have to monitor the control leg to ensure that it continues to generate spikes during the entire experiment. If the control leg stops generating spikes, you have to stop the experiment and/or move on to a new set of legs. Why is this control monitoring so important, and why do you have to stop the experiment once it stops generating spikes?

3. After repeating this experiment a few times, you can find an “average time for the spikes to go away” and an “average time for the spikes to come back”. Are they different or the same? Why?

4. You’ve probably figured out that the leg eventually dies from drying out. Is there a way you can keep the leg moist, while also allowing oxygen to pass through? Is there any material that is permeable to air but not water?

Experiment 10: Conduction Velocity

OVERVIEW

In this lab you will:

- Learn how to measure the speed of action potentials using a new earthworm preparation
- Learn about the difference between sparse coding and rate coding
- Learn how to do multiple recordings in an animal

OBJECTIVES

Before doing this lab you should:

- Review the concept of conduction velocity
- Study the anatomy of the earthworm

After doing this lab you should be able to:

- Be able to design an experiment measuring conduction velocity in different earthworm preparations under different conditions
- Understand the two ways that animals increase their conduction velocity

MATERIALS

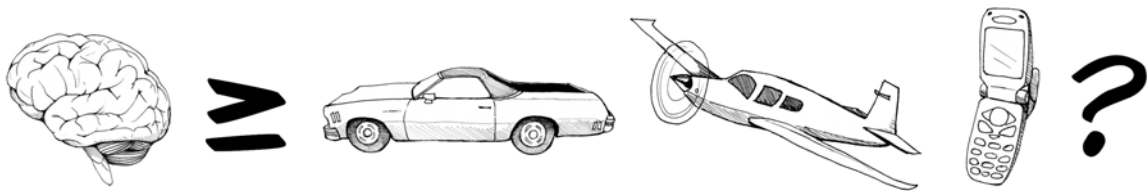
- 2-Channel SpikerBox
- Earthworm
- A Faraday Cage
- Laptop with stereo line-input
- Patch/Laptop Cable
- Ruler
- Balsa Wood to place earthworm on and insert electrode pins into

How fast are your neurons?

Background

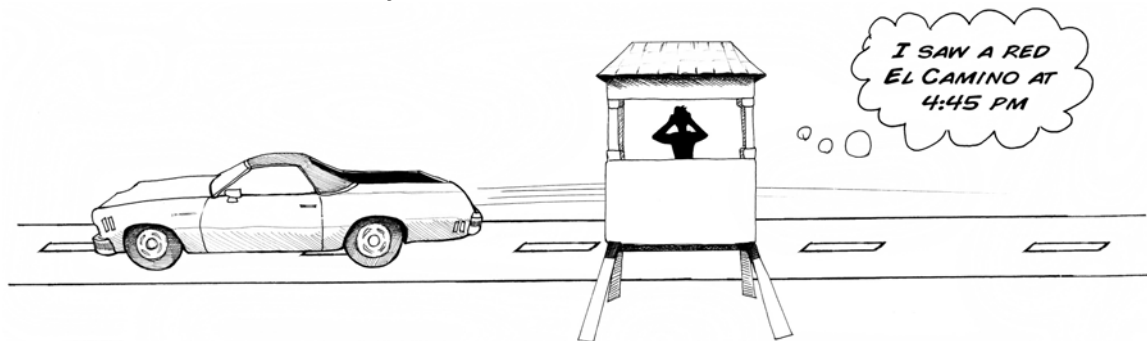
Up to this point we have been studying spikes emitted from crickets and cockroaches, mostly by monitoring the “spike rate” and “spike presence” in response to certain stimuli or conditions. We now will study “spike speed.”

You probably think the nervous system is pretty fast. You seem to hear the spikes immediately when you touch the leg of the cockroach or blow on the cerci of the crickets. But is it instantaneous? Of course not! Not even light, the fastest signal in the universe, travels instantaneously. But how fast is a nervous system? Is it faster than a car, faster than a jetplane, or faster than a cell phone? And how can we measure it?



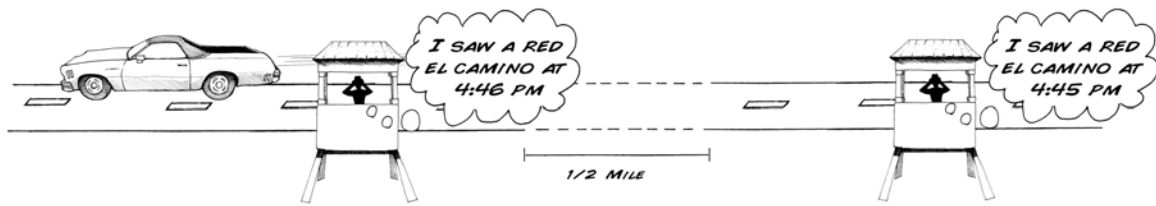
In all previous experiments, we’ve only recorded our neurons using one channel (meaning we use only one recording electrode and one ground). To measure speed (velocity) though, you need to measure both time (when a spike occurred) and distance (how far a spike has travelled down a nerve).

Take the analogy of a car on a highway. If you were looking out of a small observation hut by the road, you could tell what whether you saw a car, what kind of car it was, and the time that you saw it.



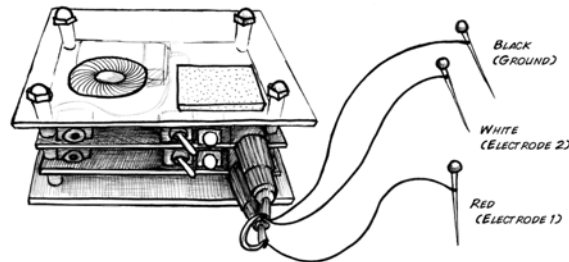
Similarly with your SpikerBox, you can currently tell if you saw a spike, perhaps what kind of neuron generated that spike (we will discuss this in a later experiment), and the time you saw the spike, but you can’t tell how fast the spike was travelling down the nerve.

Let’s go back to the car on the highway. Suppose you had a friend ½ mile down the road in a similar hut:



Later, you two could compare notes to determine the speed of the car. 1 minute = 0.016 hours. Dividing $\frac{1}{2}$ mile by 0.016 hours = 31.25 mph.

Thus, we can measure the speed with two observers, and that's why we use the "2-Channel SpikerBox" to measure two points along a nerve as a spike travels down it.



So, why don't we take our two-channel SpikerBox with our two electrodes and ground, put it in the cockroach, and measure the spike output of the two channels? You will notice immediately that there are a lot of spikes happening, in fact, way too many to keep track of it all.

Let's go back to our analog of the road. Imagine a very busy, fast moving street with many similar looking cars, say, Lakeshore Drive in Chicago, and you and your friend can only set up stations very close to each other.

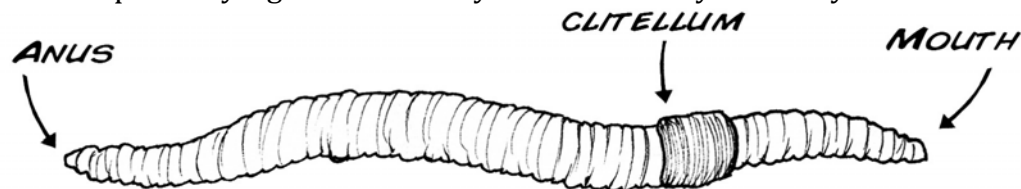


You can see the problem, there are a lot of spikes occurring in the cockroach leg, and identifying unique ones with two observers is very tricky. The femur of cockroach leg has 2 nerves inside, and inside each nerve are about 100-200 neurons, all firing

many spikes. We are also limited by how far we can place our electrodes from each other in the cockroach leg, as the leg is only about 8 mm long.

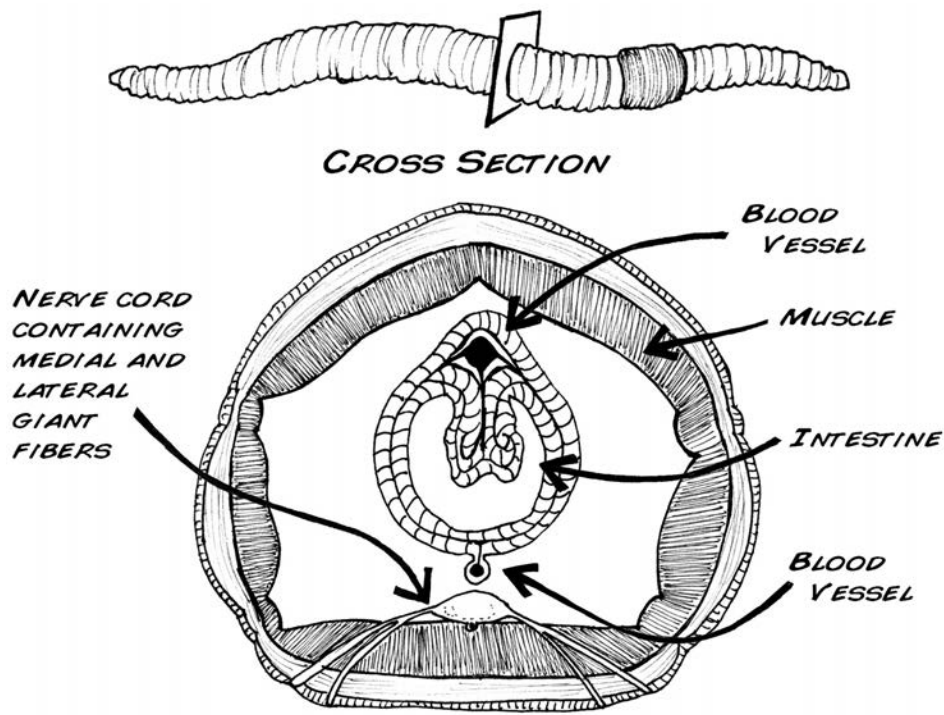
Ideally, given our limited tools, we'd want to measure spikes on a longer nerve, a nerve with only 1-3 large axons in it, and axons that do not fire many spikes.

Is there any creature in the animal kingdom that meets these qualifications? Yes! and it is probably right now under your feet and in your backyard.



TOP (DORSAL) SIDE OF WORM DARKER THAN BOTTOM (VENTRAL)

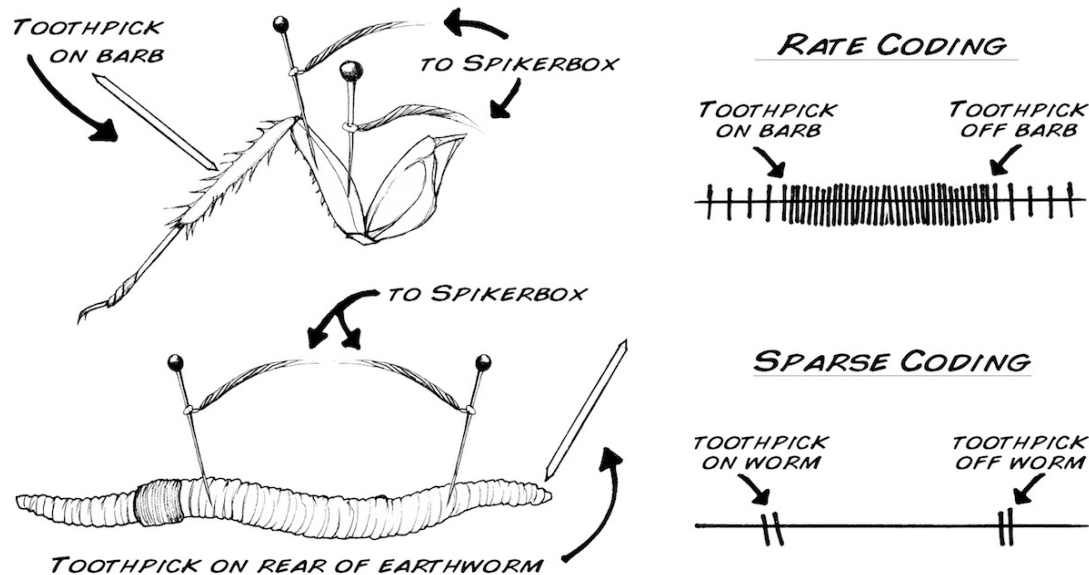
We have been studying arthropods (insects), but we now move to a new class of invertebrate: annelids! Or more commonly, worms! Enter our newest preparation: the common earthworm, *Lumbricus terrestris*. It's a simpler animal than what we've studied before, and the earthworm contains three large axons that run its length, the "medial giant" and the two "lateral giant" fibers. The medial giant fiber transmits information about the front of the worm (the part closest to the clitellum), and the lateral giant fiber transmits information from the skin cells of the posterior end of the worm (Kladt et. al 2010).



In addition to the earthworm's long length, which allows us to place our recording electrodes far apart, the earthworm also exhibits what is known as

SPARSE CODING

What is sparse coding? Let's turn back to our cockroach and the "rate coding" you have previously studied. In rate coding, the intensity of a stimulus is encoded by the rate of spikes. If the cockroach leg used a sparse coding scheme, the leg nerves would only fire 1-2 times when you touched the barb with a toothpick, and 1-2 times more when you removed the toothpick.



This sparse coding scheme is what we will see in the earthworm experiment below, and we will exploit it to measure the conduction velocity (or speed) or the spikes.

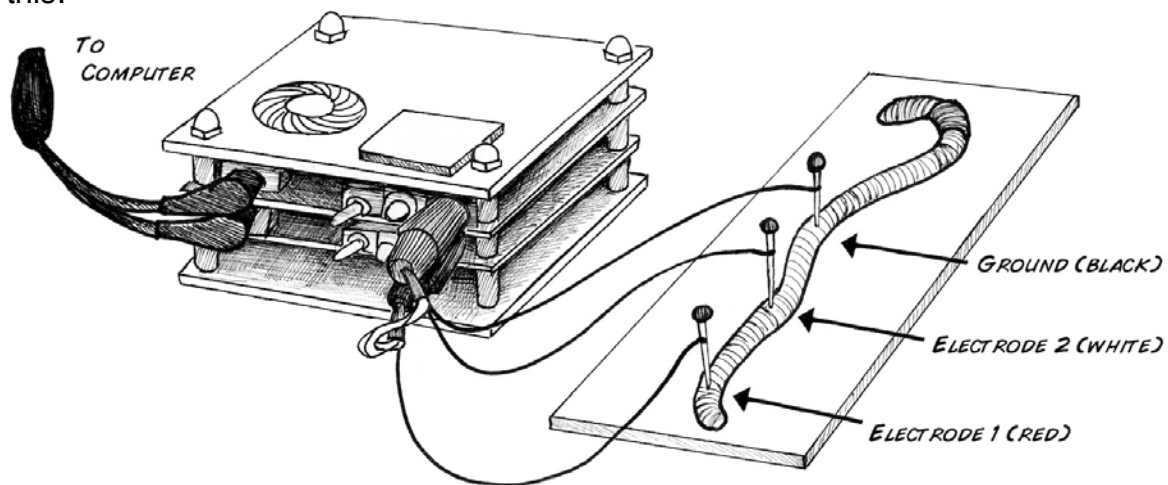
Procedure:

1. Go to your nearest pet store, sporting goods store, or gas station and purchase a box of earthworms (they are typically used to feed lizards, turtles, and fish. Fishermen use them as bait). They should be around \$3-\$4 for 12 worms. The Earthworm box should stay in the refrigerator (not the freezer) when not being used. The worms can last approximately 1-2 months.

2. Prepare a 10% ethanol solution. The easiest way to do this is to use vodka (which is normally 80 proof, or 40% ethanol). Since vodka is not much more than watered down pure ethanol, dilute it further to 1 part vodka, 3 parts water. For example, we mix 10 milliliters of alcohol with 30 milliliters of tap water. Ask your teacher to prepare this for you

3. Place a healthy earthworm in the alcohol mixture and wait seven minutes. Do not wait too long; as with human anesthesia, the delicate balance between too little anesthesia and too much is tricky. Too little anesthesia and the earthworm will move around during the experiment; the resulting muscle electrical activity (electromyogram) will drown out the small neural electrical signals you are interested in. Too much anesthesia and the nerves will not fire. We've found 7-10 minutes is a good range.

4. Place the Earthworm on a piece of balsawood or thick cork, and put your three electrodes of your two-channel SpikerBox in the posterior end of the worm, like this:



5. Place a Faraday Cage around the Earthworm, and clip the Faraday cage to the ground of either channel 1 or channel 2 of your SpikerBox (see our Faraday Cage write-up on wiki.backyardbrains.com for how to do this).

6. Turn on your 2-Channel SpikerBox, plug in your laptop patch cable to both the 2-channel SpikerBox and your Laptop, and Start the Program Audacity. Make sure your laptop line-in is capable of recording two channels. See our write-up on "testing stereo input" on wiki.backyardbrains.com for how to test this.

7. Make sure in the Audio I/O Preferences of Audacity to set your “recording device to “built-in input” for input and “Channels” to 2. Make sure “playback” is set to “Built-in Output”, and also check the “Hardware Playthrough” and “Software Playthrough” checkboxes so you can hear the spikes as you record them.

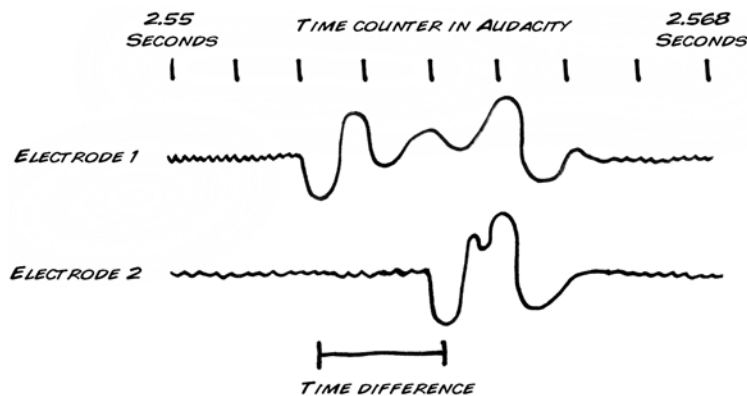
8. Press the Record Button in Audacity. You should hear some background noise, and now touch the anterior (rear) end of the earthworm with a toothpick. You should hear 1-2 faint “pops”. Those are the spikes. Interestingly, the neurons in the earthworm are myelinated (covered in insulating fat), and you will notice the spikes are much quieter than you are used to (Hartline and Coleman 2007). Many nerve diseases, such as Multiple sclerosis, are caused by a degeneration of this fatty covering.

9. Stop your Recording. Now, go back through your recording file and try to find your Spikes. You will have to zoom in multiple times to “stretch out” the signal enough to take a measurement. You should see the beginning of the spikes separated in time.

10. Using the time-axis, measure out how far apart in time the beginnings of the spikes are from each other.

11. Using a ruler with divisions in the mm range, measure the distance between electrode one and electrode two.

12. Divide the distance by the time. Viola! You have just measured conduction velocity.



Now start exploring. For example, does this measurement change from spike to spike? Does it change from earthworm to earthworm? Are smaller earthworms faster or slower than large earthworms. Is this speed sensitive to depth of anesthesia? These are all questions we would like to know, and you do to! Let us know what new discoveries you make.

Troubleshooting:

1 – If your earthworm is not healthy (not moving around the soil and not resisting/squirming when you try to pick it up), you will not get good recordings.

2 – For reasons our electrical engineering team has not been able to answer, the earthworm is a terrific antenna for electrical noise. Unless you are doing your experiment outside, you need to use a Faraday Cage for this experiment to work.

3 – You also need a laptop with a stereo (2- channel) line-input. Most laptops do, but some computers, like MacBook Airs and early MacBook Pro's, don't. Really the only to find out besides calling the laptop designers is to run a test we have designed. If you do not have a stereo line-in, you can use a USB mixer to get stereo analog inputs into your computer. We recommend the \$30 "iMic". Note that this USB interface causes a slight 1000 Hz "ring" in your recordings, but you can still successfully record earthworm spikes with this device, and sometimes you can ground the 1000 Hz ring out.

Diving Deeper

There are two ways that animals typically increase conduction velocity. In you and I, our nerves are covered with a fatty substance called myelin. In our friends the crickets and cockroaches, the neurons have larger diameters. Obvious, having large neurons limits the amount of neurons you can pack into a small space. The relationship between axon diameter and conduction speed is a function of the square root of the diameter. That is, if an axon is four times as large in diameter, the conduction velocity twice as fast. If the axon is nine times as large, the conduction velocity is three times faster.

Myelin works by decreasing the capacitance of the axonal, which decreases the time constant for recharging the membrane. This can become difficult to understand, we recommend Hartline and Coleman (2007) as a good starting reference.

Oddly, some creatures, like the Penaeidae shrimp and the earthworm you are studying today, have both large diameter axons and myelination. In the Penaeidae shrimp, the conduction velocity has been measured at 210 m/s, or 470 mph! But in the earthworm, we measured it at ~7.5 m/s, or 16 mph. Why is there such a difference in speed?

References:

Hartline and Coleman (2007) Rapid Conduction and the Evolution Review of Giant Axons and Myelinated Fibers. *Current Biology* 17, R29–R35

Kladt N, Hanslik U, Heinzel H-G (2010) Teaching basic neurophysiology using intact earthworms. *The Journal of Undergraduate Neuroscience Education* (JUNE), Fall 2010, 9(1):A20-A35

DISCUSSION QUESTIONS

1. We are we using alcohol to anesthetize the earthworm instead of ice water?

2. What happens if you reverse the ground and recording electrode 1?

3. What happens if you touch the anterior part of the worm (the mouth).

4. What are some advantages and disadvantages of sparse coding vs. rate coding?

Experiment 11: Faraday Cages

OVERVIEW

In this lab you will:

1. Learn about Faraday cages, and the reason that they work.
2. Consider how Faraday cages can be used practically to improve neural recording experiments.
3. Observe the steps to building a Faraday cage, and perform a test of this cage in a noisy environment.

OBJECTIVES

Before doing this lab you should:

- review the parameters of neural recording experiments (i.e. the type of signal recorded, the size of the signal, the way that that signal is amplified, etc.);
- learn about charges and electric fields, and the laws governing their interactions.

After doing this lab you should be able to:

- explain why a Faraday cage might be important to get good neural recordings;
- describe how a Faraday cage prevents an electromagnetic signal from crossing its surface;
- design and construct a Faraday cage for your own experimental use.

MATERIALS

- Metal Screen Mesh
- Wood Strips (3/16 inch by 1/4 inch basswood work well)
- Alligator Clip Cable
- Staple gun
- Heavy duty scissors ("tin snips")
- Hobby hack saw
- Ruler
- SpikerBox with Cockroach Leg
- "Noisy" power sources...computers plugged into outlets, fluorescent lights, etc.

INTRODUCTION

A **Faraday cage** is a container made of conducting material, such as wire mesh or metal plates, that shields what it encloses from external electric fields. In our experiments, a Faraday cage can be used to prevent external electromagnetic interference (**EMI, or noise**) from interfering with our neural recordings. As you know, the neural signals that we are recording are very small (on the order of microvolts), and we use our Spikerboxes to amplify these small signals to a large enough amplitude that we can hear and record them. Depending on our environment, though, there can be electromagnetic, radio, microwave, or other types of invisible emissions that can travel through the air and interact with the metal needles and wire that we use as electrodes. The metal then propagates the noise signal like an antenna into our neural recordings, interfering with or even drowning out our recordings so that all we hear, in the worst cases, is a radio station! A Faraday cage then can be used to block many of these noise sources.

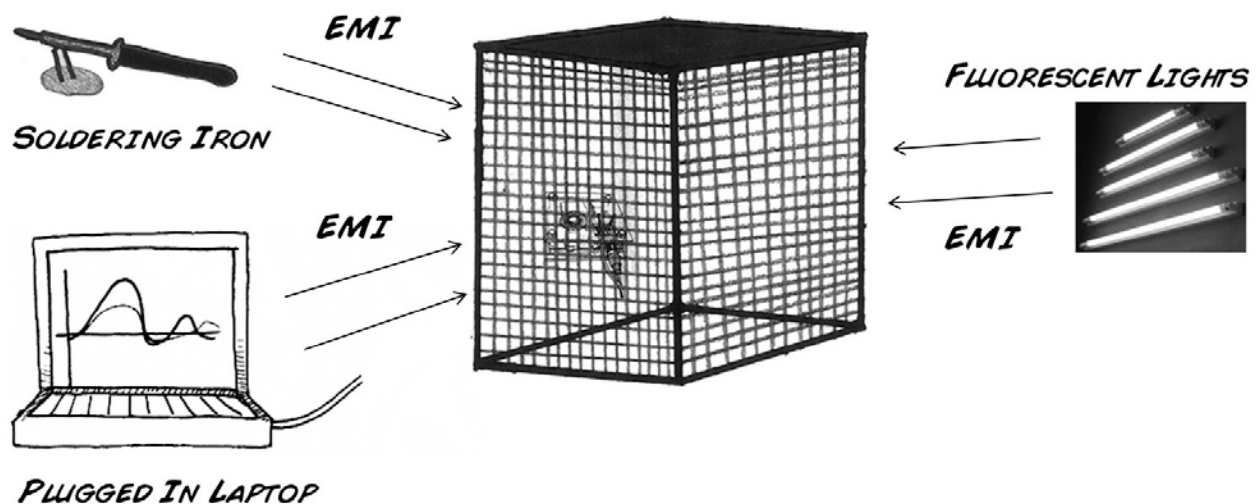


Figure 1: Faraday Cage protecting a Spikerbox from several outside electromagnetic interference (EMI) signals.

The Faraday cage is named after 1800s scientist Michael Faraday, but to learn how the cage works we start with another famous scientist, Charles-Augustin de Coulomb. Coulomb did much work on the dynamics of charged particles and the **electric fields** that they generate. Coulomb determined that the electric field, E , at a radius r away from a stationary point charge, Q , could be calculated by this equation:

$$E(r) = \frac{Q}{4\pi\epsilon_0 r^2} * e_r$$

Where ϵ_0 is the permittivity of free space and e_r is the radial unit vector.

If you don't understand the math (you will one day), it means the electric field strength declines the further away you are from an electric source. If you are driving on a highway and notice a radio station fading, for example, it's because you are getting further away from the big radio transmitter tower.

The take-home is that this law gives us the foundation for a mathematical relationship that relates charge and electric fields within a fixed volume of space. A Faraday cage encloses such a fixed volume of space, and, if the cage is made of conductive material, the cages' defining characteristic is that it prevents external charges from inducing electric fields within that volume. Here are two of the main rules that govern this barrier effect:

1. **Coulomb's Law** demands that the charges in a conductor at equilibrium be as far apart as possible, and thus the net electric charge of a conductor resides entirely on its surface.
2. Any net electric field inside the conductor would cause charge to move since it is abundant and mobile, but equilibrium demands that the net force within the conductor is equal to zero. Thus, the electric field inside of the conductor is zero.

Rule 2 tells us that the electric field inside the conductor at equilibrium is zero, and Rule 1 tells us that the charge of the conductor will be found entirely at the surface (boundary). In other words, the surface of the conducting volume becomes a barrier where charges move to and around the surface to generate fields exactly opposing any charge that seeks to cross the border, thus maintaining an interior free from external electrical interference.

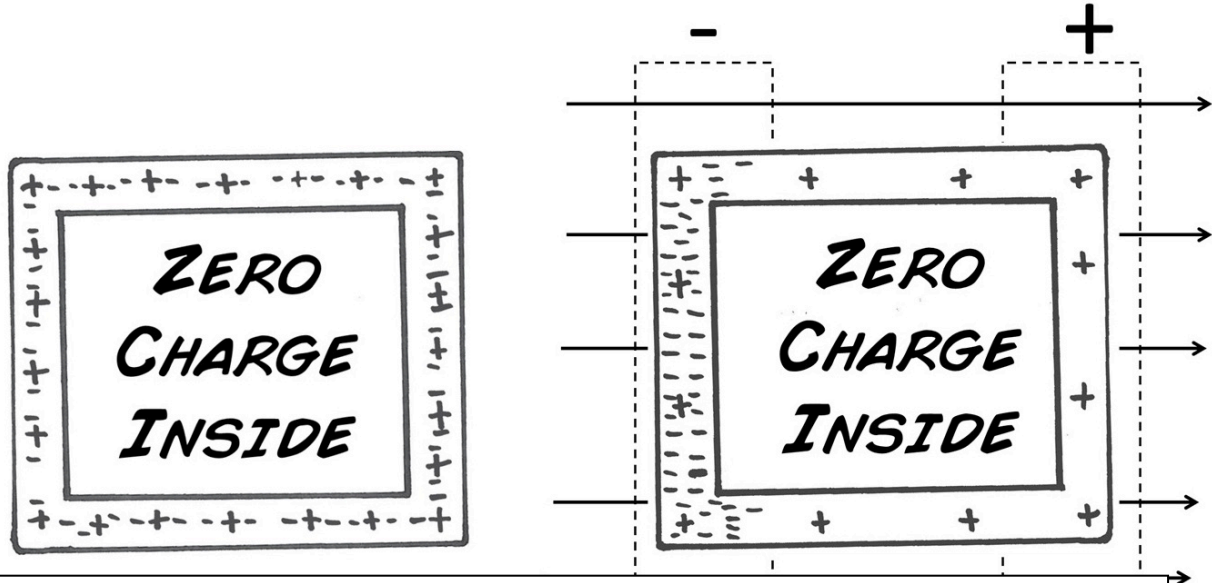


Figure 2: (left) A conducting surface at equilibrium outside of an electric field, with negative and positive charges distributed evenly throughout. (Right) the same surface in an E-field, with negative charges concentrated on left to provide a negative barrier to block the E-field from entering the surface.

Faraday first demonstrated this in a famous ice bucket and metal sphere experiment. Faraday lowered a metal ball charged with static electricity into a metal bucket supported by a wooden chair that insulated the bucket from the ground. When the charged ball was lowered into the bucket without touching the bucket, the charges on the surface of the bucket became redistributed through electrostatic induction. This concept became known as the **Faraday cage principle**.

Holes in a Faraday cage

Many Faraday cages, including the one you are building and using, have holes in them for practical purposes (to see inside the cage). Cages made in this way with fencing or mesh material still have conducting surfaces that generate the necessary barriers for electric fields, but there are types of electromagnetic waves such as radio or microwave that could theoretically enter the holes. To prevent this, the cage needs to be built with holes that are sufficiently smaller than the wavelength of these different emissions. Frequency is related to wavelength by the following equation:

$$f = \frac{c}{\lambda}$$

Where f = frequency in Hz, c is light velocity, and λ represents wavelength in meters. One rule of thumb often used for Faraday cages to prevent transmission is that the holes need to be no larger than 1/10 of the wavelength of the signal. So for a 3G cell phone that operates at a frequency of 2.1 GHz (2.1×10^9 Hz), the wavelength = $(3 \times 10^8 \text{ m/sec}) / (2.1 \times 10^9 \text{ Hz}) = .14$ meters. Thus, for a Faraday cage to prevent this noise from entering, the holes in the cage should be smaller than .014 meters (or 1.4 cm).

Using multiple layers of mesh and overlapping them such that the holes on one level are blocked by wire on another level is another effective way to build a barrier for EMI noise.

Grounding a Faraday cage

All of the theory listed above tells us that a conducting surface at equilibrium would work to prevent electric fields from entering the enclosed volume, which would thus prevent external fields from inducing currents within the volume that could show up as electrical noise in a recording. However, it should be pointed out that when external signals reach the Faraday cage, there is a transient time period when the surface charges are realigning during which **eddy currents** can form at the surface. Eddy currents are induced in conductors in response to a changing electro-magnetic fields. Eddy currents have inductance, and thus induce electromagnetic fields of their own on either side of the conductive surface. Thus, if the EMI is of high enough frequency and intensity, these eddy currents can produce field changes inside of the cage that show up in our experiments as noise.

One way to improve the EMI barrier capability of the Faraday cage is to connect the Faraday cage to a ground. The mechanisms for how this works aren't greatly understood, and experimental experience has shown that the best choice for a Faraday ground (e.g. to the wall socket, to the internal ground of the recording device, to the animal itself, etc.) could vary from experiment to experiment. Our best guess is that when the conducting surface of the cage is electrically connected to a ground source, it

keeps the entire surface tied to the same potential. Thus, with no means to induce a potential difference at the surface, external EMI is no longer can induce these transient eddy currents.

Here we will examine the effects of the Faraday cage on various conditions when doing neural recordings with the SpikerBox, along with an easy experiment you can do at home.

PROCEDURE

Exercise 1: Building a Faraday cage (video)

1. Measure out an 8 x 16 inch rectangle of screen metal mesh
2. Cut out the rectangle with heavy duty scissors
3. Measure five 8-inch lengths of wood strips
4. Carefully unroll metal mesh rectangle so that it lays flat
5. Staple wood strips through metal mesh using stapler and light-duty staples
 - a. Staple first strip at end of mesh.
 - b. Staple second strip 5.5" away from first strip, again length-wise on mesh
 - c. Staple third strip 2.5" distant from second strip
 - d. Staple fourth strip 5.5" distant from third strip
 - e. Staple fifth strip at far end of mesh
6. The wood strips are braces, so after stapling them into place, fold the mesh at each strip to form a rectangular box

Exercise 2: Testing the ability of the Faraday cage to prevent noise (video)

1. Set up a standard cockroach leg recording prep as described in Experiment 1
2. Create a noisy environment
 - a. Hook up laptop to wall outlet (60-Hz electrical noise)
 - b. Turn on soldering iron or other high power appliance.
 - c. Turn on fluorescent light
3. Turn on Spikerbox in your noisy environment
 - a. Record your observations on noise level vs. neural signal level
4. Clip an alligator clip to the ground on the Spikerbox
5. Place your Spikerbox into your Faraday cage, with cage open
 - a. Record your observations on noise level vs. neural signal level
6. Close your Faraday cage but don't connect the alligator clip to anything
 - a. Record your observations on noise level vs. neural signal level
7. Connect your alligator cable to the screen mesh of the cage
 - a. Record your observations on noise level vs. neural signal level

Exercise 3: Testing the ability of the Faraday cage to prevent cell phone activity

Microwave ovens are examples of Faraday cages, because they are meant to prevent the radiation used to cook the food from escaping into the environment. Aluminum foil is a conductive material, which may also be used to create a Faraday cage.

1. Call your cell phone and make sure that it rings (this is your control).
2. Next, take your cell phone and put it in a (turned off!) microwave.
3. Call the cell phone from another phone. Does it ring?
4. Next, open the microwave door and dial your house phone number on the cell phone. As soon as you hit 'send', shut the microwave door quickly. Does your house phone ring?
5. Finally, wrap the cell phone in aluminum foil. Call the cell phone again? Does it ring?

DISCUSSION QUESTIONS

1. What are some common "noise" sources that might interfere with a recording experiment? How might those noises enter the recording? How might you keep the noise out? Where would be the perfect place to do a recording?

2. If a Faraday cage is not connected to an external ground, does it still work? Why does it work even better when it's grounded?

3. What do you think a Faraday cage would do to an electromagnetic signal that comes from within the cage? Would someone outside of the Faraday cage be able to receive that signal?

4. What do you think would happen if you used large hole chicken wire instead of small hole screen metal mesh?

5. Why do you think cell phone text message noise still interferes with the SpikerBox even though the holes in our Faraday Cage are smaller than the 1.4 cm we calculated?
